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THE BIOSYSTEMATICS OF CARDAMINE BULBOSA (MUHL.) B.S.P. AND C. DOUGLASSII BRITT.

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In his classic monograph of the genus *Cardamine,* Schulz (1903) considered *C. douglassii* to be a variety of *C. bulbosa.* Since then, most taxonomists, including Deam (1940), Fernald (1950), Gleason (1952), Gleason and Cronquist (1963) and Stuckey (1962) have considered them to be two separate species. However, the difficulty in distinguishing these two taxa is well documented in the literature.

Schulz (1903) split *Cardamine rhomboidea,* including *C. douglassii* and *C. bulbosa,* into two major varieties. Variety *pilosa* was characterized by small amounts of pubescence at the base of the stem and on the leaf margins while variety *hirsuta* had pubescence covering the whole plant including the sepals. Each variety was further subdivided into two sub-varieties based on petal size. These varieties also seemed to vary in leaf shape causing Schulz (1903) to recognize three distinct forms. In none of these forms or varieties was flower color or length of pubescence mentioned. Although the name *C. douglassii* and its synonyms (Torrey, 1822; Gray, 1848; Britton, 1889) was known to Schulz (1903), he ignored the taxon itself. Most,

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but not all, of the purple flowered plants which Schulz observed were considered as the hirsute variety with large flowers. Most of the white flowering plants were considered as the pilose variety with small flowers.

Fassett (1940) considered the basal leaves of *Cardamine bulbosa* to be longer than wide and diagnostic in separating that taxon from *C. douglassii*. While there appears to be too much overlap between the two taxa for this to be a good distinguishing character (Stuckey, 1962) it may be used at times. The presence of a red-purple cast to the underside of the basal leaves of *C. douglassii* and the usual lack of this pigmentation on the leaves of *C. bulbosa* has also been considered as a diagnostic character (Stuckey, 1962). However, in this study it was found that any characters involving basal leaves should be avoided since by flowering time both species, especially *C. bulbosa*, lack their lower leaves. It was also noted that under conditions of high light intensity both species have purple undersides of the basal leaves.

Deam (1940) and Easterly (1967) found the length of the pod and the length of the beak too variable to be of taxonomic value. The external morphology of the seeds reveals no reliable differentiating characters separating the two species (Murley, 1951).

Several workers (Gleason & Cronquist, 1963; Easterly, 1964, 1965) have separated these two taxa by flower color with *Cardamine bulbosa* usually having white flowers (Farwell, 1925; Deam, 1940; Stuckey, 1962). Hitchcock and Standley (1919) and Stuckey (1962) report that sepal color is less variable than petal color and, therefore, a better character.

According to Braun (personal communication) these taxa are ecologically separated. However, Stuckey (1962) has found both taxa blooming at different but overlapping times, and growing sympatrically in association with supposed interspecific hybrids.

The ranges of *Cardamine bulbosa* and *C. douglassii* are quite similar in the northern areas. The northern boundary

for both appears to be New Hampshire, southern Ontario to central Michigan, and southern Minnesota (Fernald, 1950). *Cardamine bulbosa* extends west to eastern South Dakota, Kansas and Missouri, and as far south as Texas and central Florida. *Cardamine douglassii,* however, is more northern in that its southern range is from Missouri to Tennessee and Virginia. According to A. J. Sharp (personal communication), *C. douglassii* is rather rare in Tennessee and seems to be limited to calcareous soils in that region.

Stuckey (1962) concluded that these two species could best be distinguished by a combination of characters, including their different flowering periods, pubescence length, sepal color, number of branches, number of cauline leaves, and height to the lowest pedicel. He suggested that the best morphological character for separating these two taxa is the length of the stem pubescence.

Stuckey has since considered separating *Cardamine bulbosa* into two varieties as did Schulz (1903), based on the amount of pubescence (Stuckey, personal communication). He has plotted the distribution of the two forms in Ohio (unpublished data, 1966) and found that the pubescent forms are more common in eastern and southern Ohio while the near glabrous forms are found most often in northern and western Ohio.

If separate taxa are to be recognized, Stuckey's investigations (1962, 1967) have clearly established the correct nomenclature for these two species as *Cardamine bulbosa* (Muhl.) B.S.P. and *C. douglassii* Britton. Nonetheless, the question remains as to whether these are indeed two distinct taxa. In an attempt to answer this question we initiated an investigation into the biosystematics and evolution of *C. bulbosa* and *C. douglassii.*

CHEMOSYSTEMATICS

The distributional pattern of the flavonoid glycosides in both *Cardamine douglassii* and *C. bulbosa* was examined

as a possible means of differentiating between taxa and detecting cryptic phenotypic differences. A search of the literature has not revealed any previous chemosystematic work on any of the North American members of *Cardamine* or *Dentaria*. However, Grouville, Egger and Pacheco (1970) have identified Kaempferol glucosides in *C. pratensis* of Europe.

Two dimensional paper chromatography was applied to populations of both *Cardamine douglassii* and *C. bulbosa* as a possible means of differentiating between the two taxa (Table 1). Three to five (about 0.2 g) air dried cauline leaves from mature plants were soaked overnight in 50% acidified aqueous methanol. The total methanolic extract was then spotted directly onto Whatman No. 3 MM paper (46 × 57 cm). Sixty-two chromatograms representing 14 populations were included in this study (Hart, 1972; Appendix 5).

The descending method was used to develop the chromatograms. They were first developed in the long direction using tertiary-butyl alcohol, acetic acid, and water (3:1:1, v/v; TAW) and then in the short direction in acetic acid and water (5:95, v/v; HOAc) (Mabry, Markham & Thomas, 1970). After drying, the chromatograms were examined under transmitted long wave ultraviolet light with and without ammonia vapors. Most flavonoid glycosides appear as dark purple light absorbing spots in ultraviolet light and fluoresce yellow to yellow-green with ammonia vapor in ultraviolet light (Mabry, Markham & Thomas, 1970).

Partial characterization of the flavonoids was attempted with Benedict's reagent which distinguishes between monohydroxy and orthodihydroxy flavonols by forming a copper complex between adjacent hydroxyls (Seikel, 1962). This flavonol-copper complex appears dark in reflected ultraviolet light while flavonoids lacking adjacent hydroxyls appear as various shades of yellow to yellow-green.

Hydrolysis and analysis of methanolic extracts for glycoflavone (O-glycosyl flavonoid) determination followed

TABLE 1. POPULATIONS STUDIED MORPHOLOGICALLY (M), ECOLOGICALLY (E), AND CHEMICALLY (C)

Species	Location and characteristics	(M)	(E)	(C)
*C. bulbosa**	Mammoth Cave, Kentucky growing in cold spring water	1	L	Race A
C. bulbosa	Pulltight Springs, Missouri growing in cold spring water	2		Race B
C. bulbosa	Buckeye Lake, Ohio edge of floating acid bog	3	U	
C. bulbosa	Blue Ash, Ohio (near Cincinnati) swamp and moist woodland	4	P	Race C
C. bulbosa	Hueston Woods State Park, Oxford, Ohio; moist woodland *C. douglassii* nearby	5	H	Race A
C. bulbosa	Madeira, Ohio (Cincinnati Country Day School) wet woodland	6	O	Race C
C. bulbosa	Spring Valley, Ohio swamp	7	Q	Race C
C. bulbosa	Spring Valley, Ohio open marsh	8	R	Race C
C. bulbosa	Cedar Bog near Springfield, Ohio swamp (northern tendencies)	9	T	Race A
*C. bulbosa**	Mammoth Cave, Kentucky moist woods (very dry when compared to other *C. bulbosa* habitats)	10	N	
C. bulbosa	Pymatuning State Park, Andover, Ohio	11	V	
C. bulbosa	Bowling Green, Ohio; wet woodland (large numbers of *C. douglassii* nearby and putative hybrids)	12	A	Race A
C. bulbosa	Mammoth Cave, Kentucky drainage from a cold spring (Cooper Springs)		M	
C. bulbosa (Hybrid)	Camp Hook, Franklin, Ohio swamp (*C. douglassii* nearby)		S	

Species	Location and characteristics	(M)	(E)	(C)
C. bulbosa	Madeira, Ohio (Cincinnati Country Day School) mowed (periodically) moist field not used in analysis of woody plants		W	
C. douglassii/ *C. bulbosa*	Bowling Green, Ohio wet woodland (HYBRIDS)		B	Race A
*C. douglassii**	Bowling Green, Ohio; wet woodland (some *C. bulbosa* nearby with putative hybrids)	13	A	Race A
C. douglassii	Camp Hook, Franklin, Ohio wet to moist woodland (*C. bulbosa* nearby, one putative hybrid found)	14	C	Race A
*C. douglassii**	Bedford, Ohio moist woodland	15	E	
C. douglassii	Spring Valley, Ohio woodland along lake shore *C. bulbosa* nearby	16	F	Race A
C. douglassii	Rush Run Wildlife Area, Somerville, Ohio disturbed woodland	17	G	
C. douglassii	Hueston Woods, Oxford, Ohio moist woodland (*C. bulbosa* nearby)	18	H	Race A
C. douglassii	John Bachelor Preserve, Oxford, Ohio; moist-dry woodland (*C. bulbosa* and one clump putative hybrids found)	19	J	Race A
C. douglassii	Spring Valley, Ohio moist lake shore *C. bulbosa* nearby	20	K	
C. douglassii	Camp Hook, Franklin, Ohio wooded hillside (*C. bulbosa* at bottom of hill)	14	D	
C. douglassii	John Bachelor Preserve, Oxford, Ohio; wooded hillside		I	

(12) this population was not included in the morphological study
* $n = 48$, all others presumably $n = 32$

Wagner's (1966) method and standard spectroscopic methods were used for the characterization of key compounds (Mabry, Markham & Thomas, 1970; for details see Hart, 1972).

The chromatographs representing the fourteen populations of *Cardamine douglassii* and *C. bulbosa* studied reveal eighteen putative flavonoid glycosides. These spots are numbered arbitrarily in Figure 1. The composite chromatograph (Fig. 1) also reveals that the spots can be segregated in such a fashion as to reveal three or four distinct categories or races.

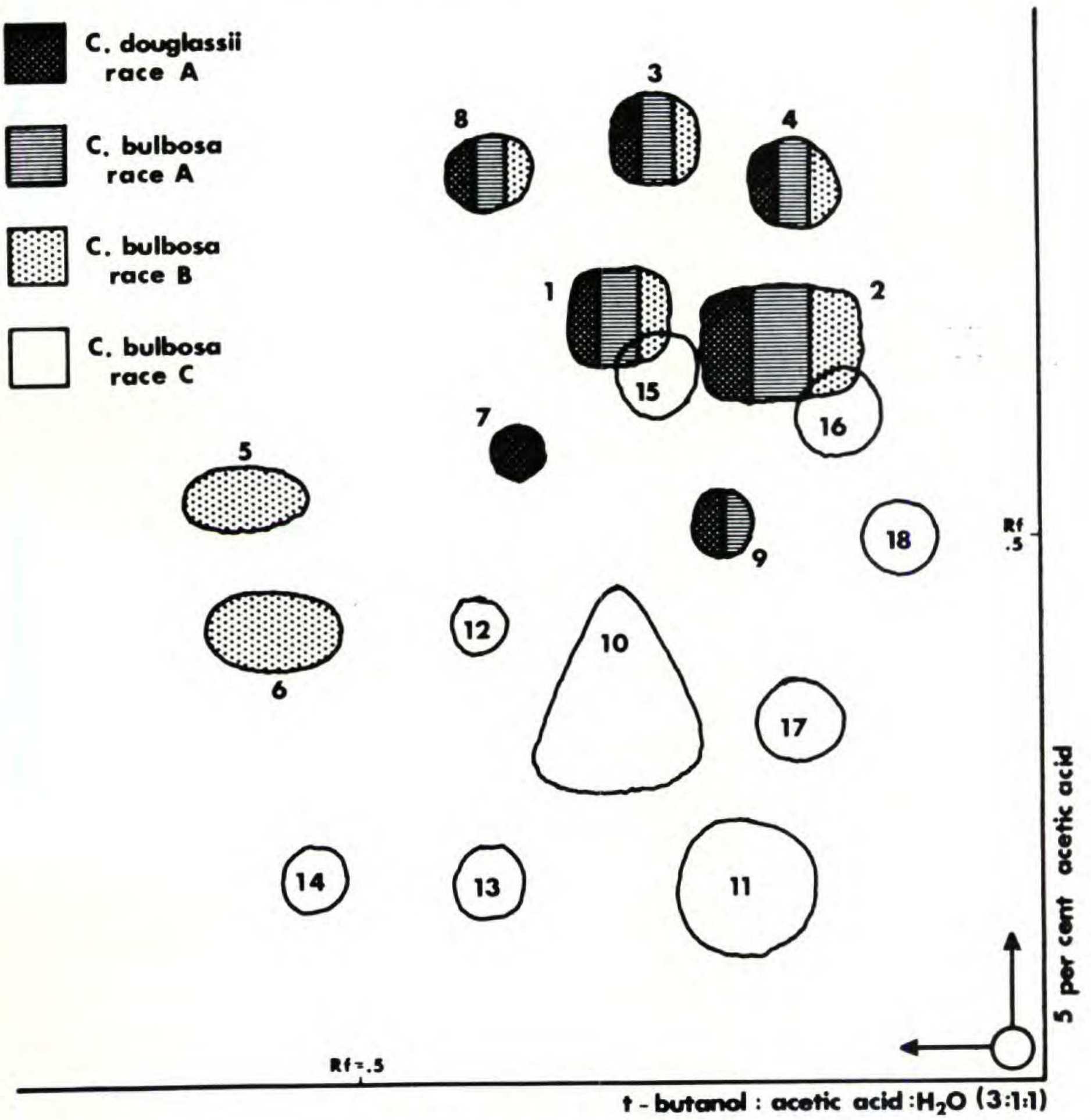

Fig. 1. Diagrammatic sketch of the chromatographic patterns shown by the chemical races in *Cardamine bulbosa* and *C. douglassii*.

Absorption maxima and colors for the spots studied have been characterized (Table 2). These data indicate that they are indeed flavonoids. Acidic hydrolysis of the compounds is easily accomplished indicating that all spots studied are flavonoid O-glycosides. Authentic samples of a few flavonoids were available for comparison with the experimental compounds. Mabry, Markham and Thomas (1970) illustrated a large number of flavonoid spectra obtained by these techniques which reinforced the identification of these compounds.

Spot 10, which is found in very large amounts in the populations of *Cardamine bulbosa* (Race C), is tentatively identified as a Quercetin 3 glycoside. The compound appears dark in Benedict's indicating two hydroxyls on the B-ring. The presence of 7-hydroxyl is shown by the bathochromic shift of Band II in sodium acetate. Both Band I and II show a shift in sodium hydroxide which is indicative of a free 5-hydroxyl. Since this compound is stable in alkaline solutions and exhibits a methanolic peak at 360 nm. it is a flavonol, with the sugar attached at the 3-position. Spot 17 has a similar spectrum (Table 2) and may also be a Quercetin 3 glycoside, possibly differing from spot 10 by having an additional or different sugar.

Spots 3, 4 and 8 have the same spectra in *Cardamine douglassii* and in *C. bulbosa* (Race A). Since these compounds have one hydroxyl in the B-ring (light in Benedict's solution) and a methanol Band I maxima of 349 nm. they are most likely Kaempferol derivatives. The lack of shifts of Band II in the sodium acetate spectra is evidence that the 7 positions are blocked. As the compounds do not break down in sodium hydroxide, the 3-position must be substituted. Therefore, spots 3, 4 and 8 are tentatively identified as Kaempferol 3-7 glycosides which probably vary from each other in the number and types of sugar substitution. Spots are found in the same location in Race B and are probably the same compound.

Spot 11 in Race C has tentatively been identified as luteolin-7-glycoside. This compound is dark in Benedict's

solution indicating the presence of two hydroxyls on the B-ring. The lack of a shift of Band II in sodium acetate is indicative of a blocked 7-position. The methanolic Band I maximum of 340 nm. indicates that the compound is a flavone.

The *Cardamine bulbosa/C. douglassii* complex may, under further investigation, turn out to be highly variable chemically. Present work reveals as many as three chemical races present among the plants studied (Fig. 1). The absence of one or two spots may, in this preliminary study, be looked upon as inconclusive. Since only 62 chromatograms were included in this study with as few as five chromatograms examined in one race, the lack of a spot may be due to its presence in a small undetected concentration.

All *Cardamine douglassii* populations studied and many midwestern *C. bulbosa* populations (Race A) appeared to have similar patterns of flavonoid constituents. One additional spot (no. 7) may be present in *C. douglassii;* however, its absence in *C. bulbosa* (Race A) has not been satisfactorily substantiated.

It is assumed that compounds 1 and 2 are different from compounds 15 and 16. This is indicated for compounds 1 and 15 (Table 2) which shows that the aglycones derived from spot 1 in *Cardamine douglassii* and *C. bulbosa* (Race A) have similar aglycone spectra while the aglycone from compound 15 of *C. bulbosa* (Race C) appears different. A similar situation occurs with compounds 2 and 16 (Table 2). In addition, *C. bulbosa* (Race C) differs from the other races in the presence of 7 dark absorbing compounds having a placement completely different from the spots in the other races (Fig. 1). Spot 10 (Quercetin 3 glycoside) of *C. bulbosa* (Race C) is always very concentrated, so much so that it normally stains the paper yellow in white light.

Cardamine bulbosa (Race B) is characterized by the presence of two dark absorbing compounds (Fig. 1) not found in the other races (nos. 5 and 6). Spectral analysis

TABLE 2. DIAGNOSTIC CHROMATOGRAPHIC ATTRIBUTES AND ABSORPTION MAXIMA OF FLAVONOID CONSTITUENTS ISOLATED FROM *CARDAMINE BULBOSA* AND *C. DOUGLASSII*

Spot No.	Race	COLOR UV	COLOR UV+NH_3	COLOR Benedicts	SPECTRA (nanometers) METOH	NaOAc	NaOH	$AlCl_2$
1	C.d. – A	D	YG – G	? *	257,264s,327s,367	257,264s,327s,367	267s,330s,424	
	C.b. – A	D	YG – G	? *	254,264s,324,367	254s,264s,329s,368	269,355,449	
	C.b. – B	D	YG – G	? *	250,267s,325,371	245,267s,331s,370	264s,348s,352s,412	
2	C.d. – A	D	YG – G	D *	248,280s,393,365	246,280s,301	246,281,292,330	247,295,324,344
	C.b. – A	D	YG – G	D				
2	C.b. – B	D	YG – G	D				
3	C.d. – A	D	YG	L	266,330s,349	267,352,402s	275,273s,343s,393	276,296s,345,394
	C.b. – A	D	YG	L	266,330s,349	267,352,402s	275,273s,343s,393	276,296s,345,394
3	C.b. – B	D	YG	L				
4	C.d. – A							
4	C.b. – A	D	YG	L	266,330s,349	267,352,402s	275,273s,343s,393	276,296s,345,394
4	C.b. – B	D	YG	L				
5	C.b. – B	d	yg	D				
6	C.b. – B	d	yg	D				
7	C.d. – A	d	y	L				
8	C.d. – A	D	YG	L	267,330s,349	267,352,403s	275,273s,343s,392	276,297s,345,394
	C.b. – A	D	YG	L	267,330s,349	267,352,402s	276,273s,342s,393	276,297s,345,392
	C.b. – B	D	YG	L				
9	C.d. – A	d	y	D				
	C.b. – B							

10	C.b. – C	(D)	YG – G	D	257,303s,360	272,327s,375	272,328s,410	273,297s,368s,413
11	C.b. – C	D	y	D	256,307s,340	257,266s,332	272,400	269,305s,334
12	C.b. – C	D	YG	D				
13	C.b. – C	D	YG	D	256,270s,302s,334	275,327,385	270,389	
14	C.b. – C	D	y	D				
15	C.b. – C	D	YG – G	? *	262s,290,367	232,290	237,322	273,301s,330,409
16	C.b. – C	D	YG – G	D *	267,295s,325s,367			
17	C.b. – C	D	YG	D	257,274s,301s,361	274,324s,385	273,331s,414	274,299s,363s,416
18	C.b. – C	D	y	D				

* Spectra are of aglycones; all others are glycosides.

RACE
- C.d. – A *C. douglassii*
- C.b. – A *C. bulbosa* – Race A
- C.b. – B *C. bulbosa* – Race B
- C.b. – C *C. bulbosa* – Race C

UV
- (D) Very dark spot
- D Dark spot
- d Faint dark spot

$UV + NH_3$
- YG Bright yellow green
- yg Faint yellow green
- G Bright green
- y Faint yellow

BENEDICTS
- D Dark in reflected UV light with Benedicts Reagent
- L Light in reflected UV light with Benedicts Reagent

SPECTRA (nanometers)
- s Shoulder

of these was not attempted because of the few plants available with which to work. The specimens of Race B came from Pulltight Springs, Missouri, at the type locality of *C. bulbosa* f. *fontinalis* Palmer & Steyermark (1938). The plants were found growing almost totally submerged in cold spring water and therefore differ ecologically from all other populations studied.

Within the limits of the flavonoid chemical data available, *Cardamine douglassii* appears to be one of three chemical races of a highly variable species complex. The wide range of flavonoid chromatographic patterns in *C. bulbosa,* with the slight variation of one of these patterns being *C. douglassii,* may be evolutionally significant if one can assume that a highly variable taxon is likely to be more primitive than a less variable one (Davidson & Dunn, 1967). There is a possibility of finding morphological characters or certain ecologic factors associated with the different chromatographic patterns. This will be discussed further in the section on morphology. The geographic distribution of the chemical races and the possible reasons for this distribution needs further study.

CHROMOSOME NUMBERS

The base chromosome number for *Cardamine* has been reported to be both seven and eight by Lövkvist (1956) in his investigation of the high polyploid *C. pratensis* (Eucardamine) complex. Lewis *et al.* (1962) reported a chromosome number of $n = 32$ in *C. bulbosa* from Texas (base number = 8). Haploid counts of $n = 28$ and $n = 56$ (base number = 7) have also been reported (Easterly, 1963) from both *C. douglassii* and *C. bulbosa* in a population of plants near Bowling Green, Ohio. In an effort to resolve the conflict in the reported chromosome number for *Cardamine douglassii* and *C. bulbosa,* a cytological investigation was made of several populations of these taxa (Table 3).

Young flower buds were killed and fixed in Carnoy's solution. Snow's acid carmine method was used to stain the meiotic material which was squashed and subsequently

TABLE 3. SUMMARY OF HAPLOID CHROMOSOME NUMBERS AND PERCENT OF POLLEN STAINABILITY WITHIN *CARDAMINE BULBOSA* AND *C. DOUGLASSII*

Species	Population Number	No. of Plants	No. of Counts	Haploid Chromosome Number (n)	Pollen Stainability Average/300 Grains
C. bulbosa	8	7	27	32	98.4
C. bulbosa	9	5	13	32	
C. bulbosa	2	1	3	32	64.9
C. bulbosa	12	4	6	32	82.9
		1	2	32	
			1	40	
		1	7	±40	
		2	3	48	
C. bulbosa	1	4	11	48	
C. douglassii	16	2	3	32	72.3
C. douglassii	19	4	7	32	
C. douglassii	14	1	1	±30	
C. douglassii	18	2	3	32	92.1
C. douglassii	13	10	27	48	99.1
		1	2	48	
			1	32	
C. douglassii	15	2	4	72	
		1	3	48	

mounted in Hoyer's medium (Snow, 1963; Beeks, 1955). Since it was not possible to examine and count all the material within the week it was collected, selected samples were stored, either stained or unstained, in a freezer in 70% ethyl alcohol, for future study.

Meiosis occurs in *Cardamine douglassii* between the beginning of December and early March while it does not occur in *C. bulbosa* until late March and April. This appears to be an important species character; however, it is of little use in field or herbarium identification.

Chromosome counts proved extremely difficult to make as the chromosomes are small and separate with great difficulty. The large majority of plants studied produced no acceptable counts due to either poor separation or a lack of meiotic material.

The most common chromosome number in both species is $n = 32$ (Fig. 2-4) which is found in populations in southwestern Ohio. However, some populations of both species are found to contain higher chromosome numbers. *Cardamine bulbosa,* in the Mammoth Cave populations (pop. 1, 10), possesses a haploid number of 48 (Fig. 6). This number is also found in a few plants of *C. bulbosa* in the Bowling Green population (pop. 12).

The chromosome number of the northern Ohio population of *Cardamine douglassii* differs from the more typical number ($n = 32$) found elsewhere. All plants of *C. douglassii* collected at the Bowling Green, Ohio, site have a haploid number of 48 (Fig. 5). It was also noted that *C. douglassii* from this site produced fewer seeds than produced by this species elsewhere. Plants from this population show a relatively low percentage of good crosses under greenhouse control. These two conditions may be indicative of irregular meiosis in individuals of this population. In Bedford, Ohio (pop. 15), only three plants were counted, two of which have a haploid number of 72 (Fig. 7) and the other, a haploid number of 48 (Table 3).

Two artificially produced F_1 plants (Fig. 8 and 9) resulting from a *Cardamine douglassii* ($n = 32$) × *C. bulbosa* ($n = 32$) cross have meiotic figures with a few lagging chromosomes and possibly incomplete pairing (Fig. 10) with a haploid number near 32. A similar situation was found in a greenhouse hybrid between *C. douglassii* ($n =$

Figs. 2-9. Meiotic chromosomes of *Cardamine bulbosa* and *C. douglassii.* × 1000. Fig. 2. *C. douglassii* from Hueston Woods State Park, Oxford, Ohio ($n = 32$). Fig. 3. *C. douglassii* from John Bachelor Preserve, Oxford, Ohio ($n = 32$). Fig. 4. *C. bulbosa* from Spring Valley, Ohio ($n = 32$). Fig. 5. *C. douglassii* from Bowling Green, Ohio ($n = 48$). Fig. 6. *C. bulbosa* from Three Springs, Mammoth Cave, Kentucky ($n = 48$). Fig. 7. *C. douglassii* from Cleveland Metropolitan Park, Bedford, Ohio ($n = 72$). Fig. 8. Chromosome pattern of hybrid *C. douglassii* ($n = 32$) × *C. bulbosa* ($n = 32$), ($n =$ ca. 32). Fig. 9. Chromosome number of hybrid *C. douglassii* ($n = 32$) × *C. bulbosa* ($n = 32$), ($n =$ ca. 32 + 2).


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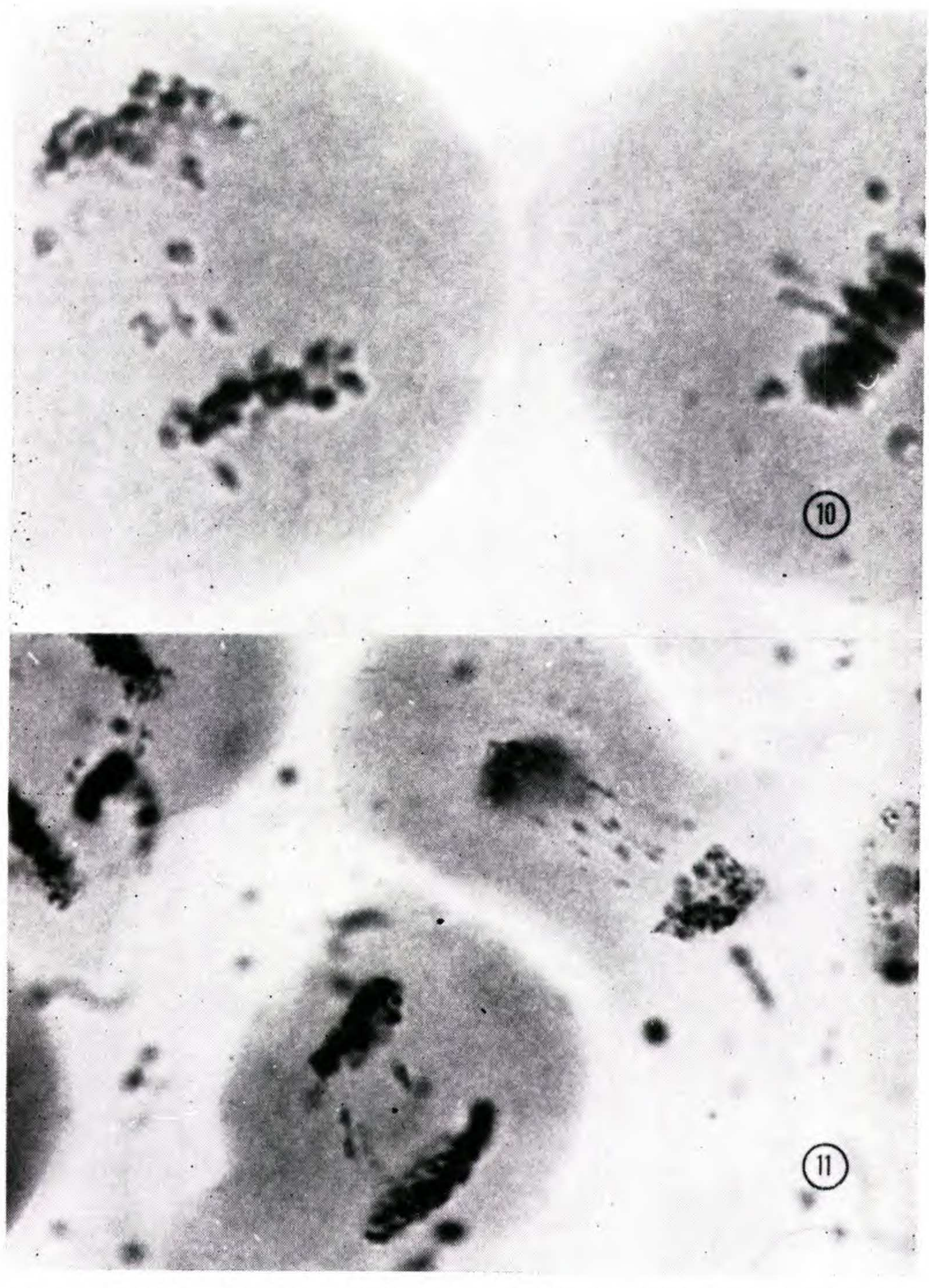


Figs. 10-11. Fig. 10. Meiosis in a hybrid, *C. bulbosa* ($n = 32$) $\times$ *C. douglassii* ($n = 48$), see text for explanation. Fig. 11. Meiosis in *C. douglassii*, Bowling Green, Ohio ($n = 48$).

48) × *C. bulbosa* ($n = 32$). Lagging chromosomes are common in the buds collected from many plants in different populations (Fig. 11). One putative hybrid collected from the Bowling Green population has a haploid number of ca. 40.

A number of plausible mechanisms could be offered here to explain the presence of the chromosome numbers $n = 48$, $n = 72$ and $n = 40$. However, a more extensive cytological investigation of more individuals and populations will have to be made before any one hypothesis can be advanced over another.

EXPERIMENTAL HYBRIDIZATIONS

The artificial pollinations and hybridizations reported here represent a breeding study undertaken to explore the importance and mechanism of genetic isolation between *Cardamine douglassii* and *C. bulbosa*. The breeding program was undertaken under uniform greenhouse conditions during two growing seasons. Reciprocal crosses between a number of populations of these two taxa were attempted and where possible each cross was undertaken several to many times. Effective emasculation prior to crossing required the removal of the androecium as well as the calyx and corolla. Pollen was transferred to a stigma directly from the anther of the male parent. Initially, the artificially crossed flowers were bagged in glassine envelopes (Kondra & Douney, 1968). However, bagging was discontinued since the self incompatible plants exhibited no outcrossing effects and failed to set seed under normal greenhouse conditions.

Crosses between populations of *Cardamine douglassii* with a haploid chromosome number of 32 show a high percentage of fruit set (85.7%) while crosses between $n = 48$ populations give a low (14.7%) percentage of fruit set. When crosses are made between populations of these two different chromosomal levels the percentage of fruit set is 46.3% when the $n = 32$ individuals are the female parent (Table 4).

TABLE 4. CROSSING RELATIONSHIPS BETWEEN SPECIES AND RACES OF *CARDAMINE BULBOSA* (B) AND *C. DOUGLASSII* (D)

Female	Male	No. of Attempts	No. of Fruits	Percent Fruit Set	Aver. No. seeds/fruit
B(race A)	× B(race A)	11	11	100	7.2
B(race C)	× B(race C)	51	40	78.4	5.3
B(BG)	× B(race A)	23	17	73.9	2.8
B(race A)	× B(race C)	10	5	50	4.2
B(race A)	× B(BG)	3	0	0	—
B(race A)	× B (n = 48)	1	1	100	1
B(n = 48)	× B(race C)	2	0	0	—
B(n = 48)	× B(BG)	3	0	0	—
B(BG)	× B(n = 48)	2	2	100	3
B(n = 48)	× D(n = 48)	4	0	0	—
B(race A)	× D(n = 32)	28	10	57.1	3.3
B(race C)	× D(n = 32)	110	31	32.7	5.9
B(race C)	× D(n = 48)	23	19	82.6	5.6
B(BG)	× D(n = 32)	23	11	60.9	2.6
B(BG)	× D(n = 48)	3	2	66.7	2.5
D(n = 32)	× D(n = 32)	14	12	85.7	5.3
D(n = 48)	× D(n = 48)	14	2	14.3	5.0
D(n = 32)	× D(n = 48)	41	19	46.3	5.8
D(n = 48)	× D(n = 32)	25	7	28	4.9
D(n = 32)	× B(race A)	24	15	62.5	8.6
D(n = 32)	× B(race C)	59	18	30.5	7.8
D(n = 48)	× B(race C)	19	0	0	—
D(n = 48)	× B(BG)	5	3	60	5.3
B(race A)	Selfed	9	0	0	—
B(race C)	Selfed	8	0	0	—
D(n = 32)	Selfed	26	1	3.8	1
D(n = 48)	Selfed	16	1	6.3	1

With the exception of the Bowling Green *Cardamine bulbosa*, Bowling Green *C. douglassii* is generally isolated from the other races when used as a female parent. Interestingly, when *C. douglassii* ($n = 48$) pollen is put on the stigma of *C. bulbosa* (chemical Race C) fruit set is 82.6 percent while in the same cross with other chemical races there is no fruit set.

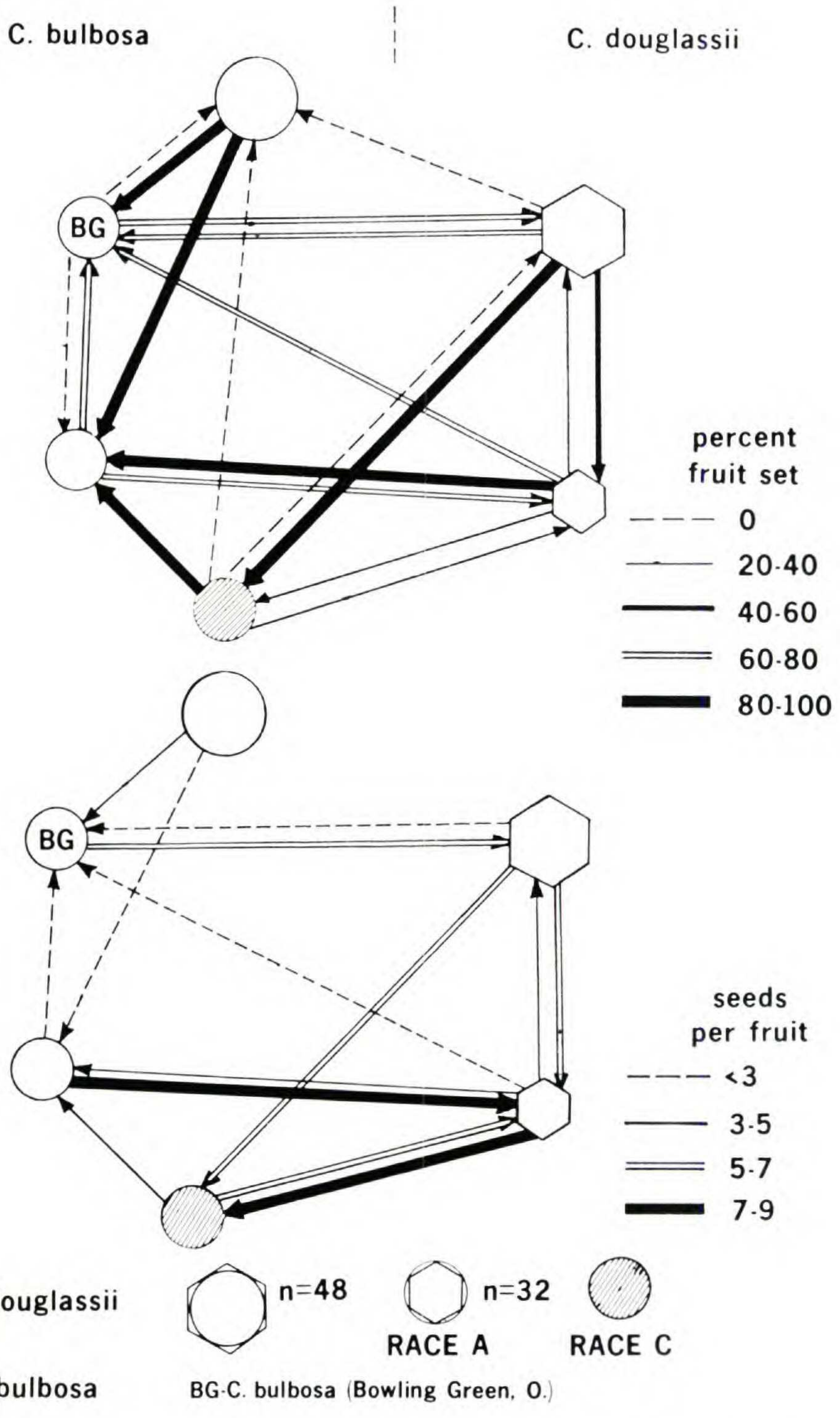


Fig. 12. Percentage of fruit set and number of seeds per fruit in intraspecific and interspecific crosses of *C. bulbosa* and *C. douglassii*.

One should note that although only a few crosses could be made, the preliminary evidence indicates that the *Cardamine bulbosa* ($n = 32$) of the Bowling Green population may act as a good female parent for all races tested, including those with a haploid number of 48 (Fig. 12). While many fruits are produced on these plants, there appears to be a decrease in the number of seeds per fruit (Table 4, Fig. 12).

Since putative hybrids have been found in the field, it is desirable to know the fertility of artificially produced, greenhouse grown hybrids for comparison with their parents. Crosses of hybrids $F_1 \times F_1$ produce fruit from 30 percent of the attempts. Of the eight F_2 plants grown to flowering size, only three actually flowered. This was unusual as all other plants raised in this study flowered with little difficulty when mature and after being vernalized at 4° C. for two months. This F_2 breakdown did not seem to affect those plants which did flower, as eight good fruits with an average of four seeds each were produced from eight attempted crosses with other F_2's (Table 5).

Backcrosses of *Cardamine douglassii* ($n = 32$) and *C. bulbosa* (chemical Race A) to the F_1's were easily made as was the reciprocal to *C. bulbosa* (Table 5). The relative ease in making backcrosses indicates that a large amount of gene flow between the two species is possible.

The chromosome race of *C. bulbosa* ($n = 48$) found in Kentucky was not studied in detail; however, from a total of fourteen crosses attempted (Table 4) it appears as though the race may behave somewhat like the Bowling Green population of *Cardamine douglassii* ($n = 48$). *Cardamine bulbosa,* having a haploid number of forty-eight, may act as the female parent only with difficulty while it serves as the male parent with ease (Fig. 12). Only four crosses of the Kentucky *C. bulbosa* ($n = 48$) and *C. douglassii* ($n = 48$) were tried, and none produced fruit.

Preliminary work demonstrates that both species of *Cardamine* are protogynous. The stigma often emerges one or two days before the flower opens and two to three

TABLE 5. CROSSING RELATIONSHIPS OF THE HYBRIDS OF *CARDAMINE BULBOSA* AND *C. DOUGLASSII*

Female	Male	No. Attempts	No. Fruit	Percent Fruit set	Average No. Seeds/Fruit
F_1 (n = 32, race A)	× F_1 (n = 32, race A)	153	56	36.6	7.1
F_1 (n = 32, race C)	× F_1 (n = 32, race A)	50	3	6.0	3.0
F_1 (n = 32, race A)	× F_1 (n = 48, race A)	8	5	62.5	1.2
F_1 selfed		20	0	0	—
F_1 (n = 32, race A)	× B (race C)	4	1	25	5
F_1 (n = 32, race A)	× B (race A)	12	12	100	8.9
F_1 (n = 32, race A)	× D (n = 32)	45	30	66.7	11.07
B (race A)	× F_1 (n = 32, race A)	31	31	100	7.5
B (race C)	× F_1 (n = 32, race A)	4	2	50	6.0
D (n = 32)	× F_1 (n = 32, race A)	12	2	16.7	5
F_2	× F_2	8	8	100	4.0
F_1 (n = 32, race A)	× F_2	4	1	25	4.0

B = *C. bulbosa*
D = *C. douglassii*
F_1 = *C. bulbosa* crossed with *C. douglassii*
F_2 = F_1 crossed with F_1
n = 32 *C. douglassii* (n = 32)
n = 48 *C. douglassii* (n = 32)

race A — *C. bulbosa* (chemical race A)
race C — *C. bulbosa* (chemical race C)

Fig. 13. *C. bulbosa* with the stigma protruding prior to anthesis, suggesting incipient protogyny.

days before the anthers dehisce (Fig. 13). Tests have shown the stigma to be receptive at this time. Protogyny has been reported in the Cruciferae and related genera before (Harriman, 1965; Johnson, 1971; Rollins, 1971).

An examination of the crossing relationships of *Cardamine douglassii* and *C. bulbosa* has revealed some interesting facts. As previously mentioned, *C. bulbosa* appears to be composed of at least three chemical races; however, while both races A and C cross among themselves and produce fruit almost 80 percent of the time (Table 4), their crossability to *C. douglassii* differs. *Cardamine bulbosa* (chemical Race A) produces fruit about 60 percent of the time when it is crossed to *C. douglassii* ($n = 32$) while *C. bulbosa* (chemical Race C) produces fruit only a little over 30 percent of the time. When fruits are produced, little difference is noted in seed production in crosses between the two chemical races and *C. douglassii*. The ability of the Race C to cross with the F_1's of the aforementioned cross is distinctly less than that of Race A.

Crossing relationships indicate possible genetic differences between the chemical races of *Cardamine bulbosa*. Chemical Race C seems slightly more isolated from *C. douglassii* than does the chemical Race A as far as the production of F_1's and backcrosses is concerned. It is possible that Race C is a later adaptation of *C. bulbosa*, less apt to hybridize with *C. douglassii*. Another possibility is that *C. bulbosa* (Race A) is a variant originating from hybridization and later backcrossing between *C. bulbosa* (Race C) and *C. douglassii*. Its geographic distribution needs further study in light of this possibility.

The chromosome race $n = 48$ of *Cardamine douglassii* has a lower fruit set than the other populations when intrapopulational crosses are made (Table 4). Data from crosses between populations having different chromosome numbers (at least in *C. douglassii*) indicate the isolation of these populations from each other. *Cardamine douglassii* ($n = 48$) does not act as a good female parent in interspecific crosses. The fact that one population of *C. bulbosa* which is sympatric with a *C. douglassii* ($n = 48$) population can cross readily with it indicates a breakdown of what genetic isolation mechanisms there are in this population. One might expect that in the Bowling Green, Ohio, population, where *C. bulbosa* ($n = 32$) and *C. douglassii* ($n = 48$) are capable of hybridizing, there would be hybrids present as well as the possibility of many backcrossed plants.

Interspecific crosses proved relatively easy to make, at least as easy as some interracial crosses within species. This indicates that genetic incompatibility is not an important isolating mechanism separating *Cardamine bulbosa* and *C. douglassii* except where different chromosome numbers exist. All artificial crosses were made in the greenhouse at Miami University. In the field, examination of putative hybrids showed that there is some fruit development but that very few seeds mature.

When hybrid crosses are made in nature, during the slight overlap in blooming time, it is most likely that the

Cardamine douglassii pollen would be transferred to the emergent stigma of *C. bulbosa*. At the time of overlap, *C. bulbosa* is just beginning to flower while *C. douglassii* flowers are wide open. Because of protogyny, the flowers of *C. douglassii* would be pollinated with *C. douglassii* pollen by this time. If pollinator inconsistency is assumed, an interspecific cross with *C. bulbosa* as the female parent would be more likely than the reverse.

Only a small difference in number of fruits set is noticed between *Cardamine bulbosa* (Chemical Race A, $n = 32$) and *C. douglassii*. Important differences, however, can be seen in number of seeds per fruit (Fig. 12). The more easily accomplished cross using *C. bulbosa* as female parent produces fruit with a few seeds while the more difficult cross using *C. douglassii* as female parent leads to the production of fruit with many seeds.

At first glance, the phenomenon of protogyny in these self incompatible species is perplexing as it cannot increase outcrossing, although it may prevent a wastage of gametes. It appears that in these two closely related *Cardamine* species protogyny is at least partially responsible for keeping these species evolutionarily separate. Johnson (1970) may have found a similar situation in *Arabis holboellii* and the closely related *A. sparsiflora*.

PHENOLOGY

Since the two species are supposedly isolated due to different flowering times, the flowering period of *Cardamine bulbosa* and *C. douglassii* was studied over two growing seasons. Starting in late March through early June, two to three visits a week were made to four different areas in southwestern Ohio. Each of these sites contained both *C. bulbosa* and *C. douglassii*. Two quadrats (ca. $10' \times 10'$) were established at each site and the number of plants of each species in bloom at each visit was noted.

Phenology of *Cardamine bulbosa* and *C. douglassii* is shown for the spring seasons of 1969 and 1970 (Fig. 14).

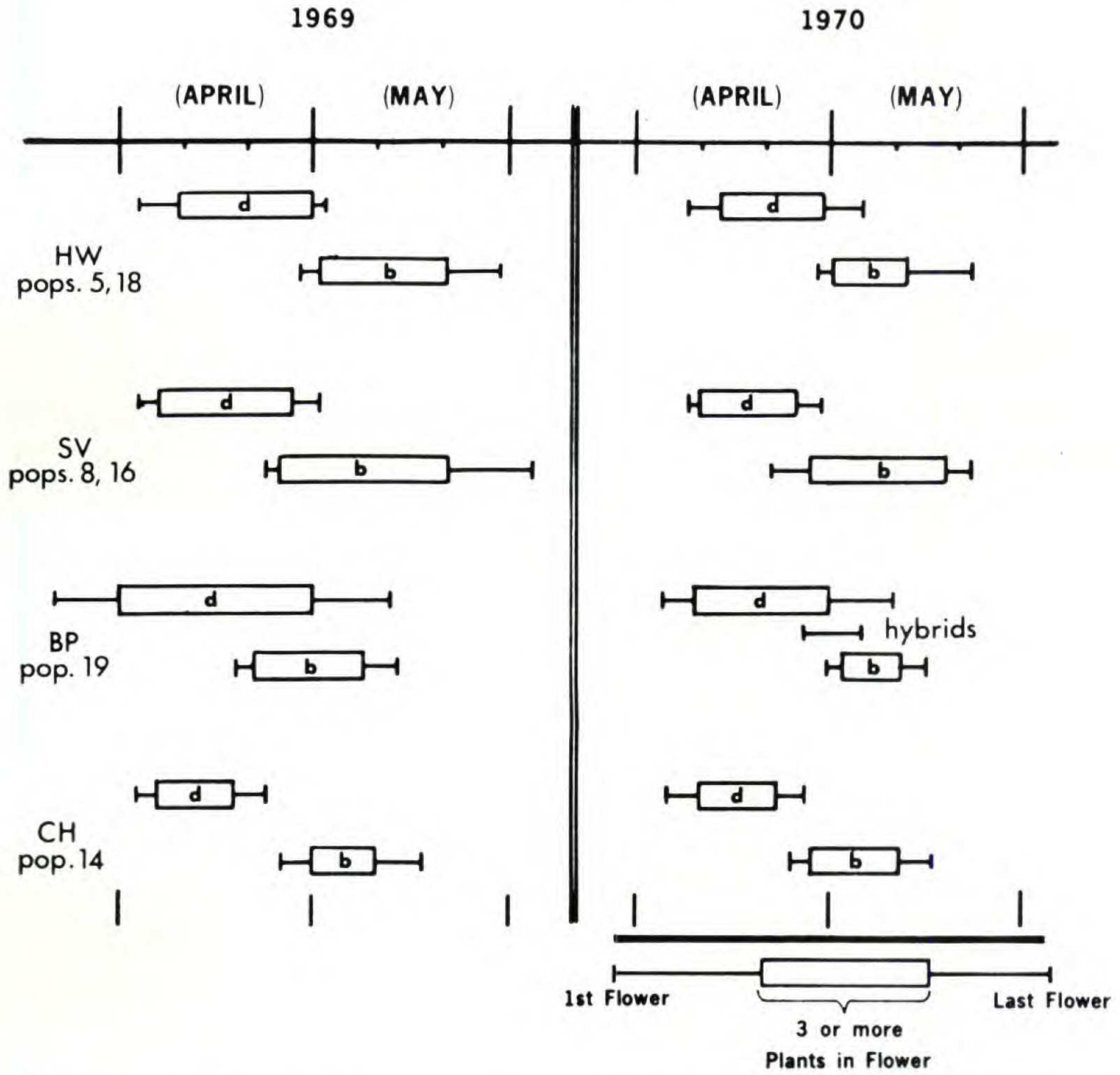


Fig. 14. Time of flowering sequence of *Cardamine douglassii* (d) and *C. bulbosa* (b) in southwestern Ohio. 100 sq. ft. quadrat.

Each rectangle in Figure 14 indicates the period of time three or more plants were in bloom in that particular quadrat. The horizontal line extending in either direction indicates the time the first and last plant of that species was in bloom in the neighboring area. *Cardamine douglassii* begins flowering around the first week of April and continues until early May. *Cardamine bulbosa*, on the other hand, begins flowering around the last week of April and may continue into June.

While these two species flower at largely different times, an overlap of flowering does occur in late April. Stuckey (1962) noticed a similar situation in Michigan. However,

due to the more northern position of that state, the overlap period occurred in mid-May. Putative hybrids (based on intermediate pubescence length and height) have been found at the phenological site on the John Bachelor Preserve near Oxford, Ohio. Putative hybrid plants at this and other locations have an intermediate flowering time between the parental types.

While *Cardamine bulbosa* and *C. douglassii* flower at different times, the overlap is such that one might expect hybrids to be produced relatively often. The overlap does vary from year to year and from site to site ranging from almost complete overlap at the John Bachelor Preserve near Oxford, Ohio, in 1969 (population no. 19), to no overlap at all at Camp Hook (Boy Scout camp) near Franklin, Ohio (population no. 14), in the same year.

POLLINATION

Hybridization in nature is mediated through the activities of pollinators. One bee visiting different species of *Cardamine* could possibly cause interspecific hybridization. In an attempt to identify the visiting insects, hopefully the pollinators, those insects visiting either *C. bulbosa* or *C. douglassii* were collected during the spring of 1969 and 1970. Collections were made on clear days since cloudy days produced little pollinator activity. Insects were netted between ten in the morning and three in the afternoon. An attempt was made to collect nearly all insects seen without creating a disturbance which might frighten certain species away. *Apis mellifera* may be over-represented due to its size. However, this was realized at the time of collection.

Pollen from the corbicular load was removed and mounted in Hoyer's solution stained with fast green. It is not easy to distinguish the pollen of the different genera of the Cruciferae, let alone separate *Cardamine bulbosa* pollen from that of *C. douglassii.* It is believed that relative abundance of crucifer pollen grains (out of sample of 300 grains) contained in the corbicular load of a bee can be

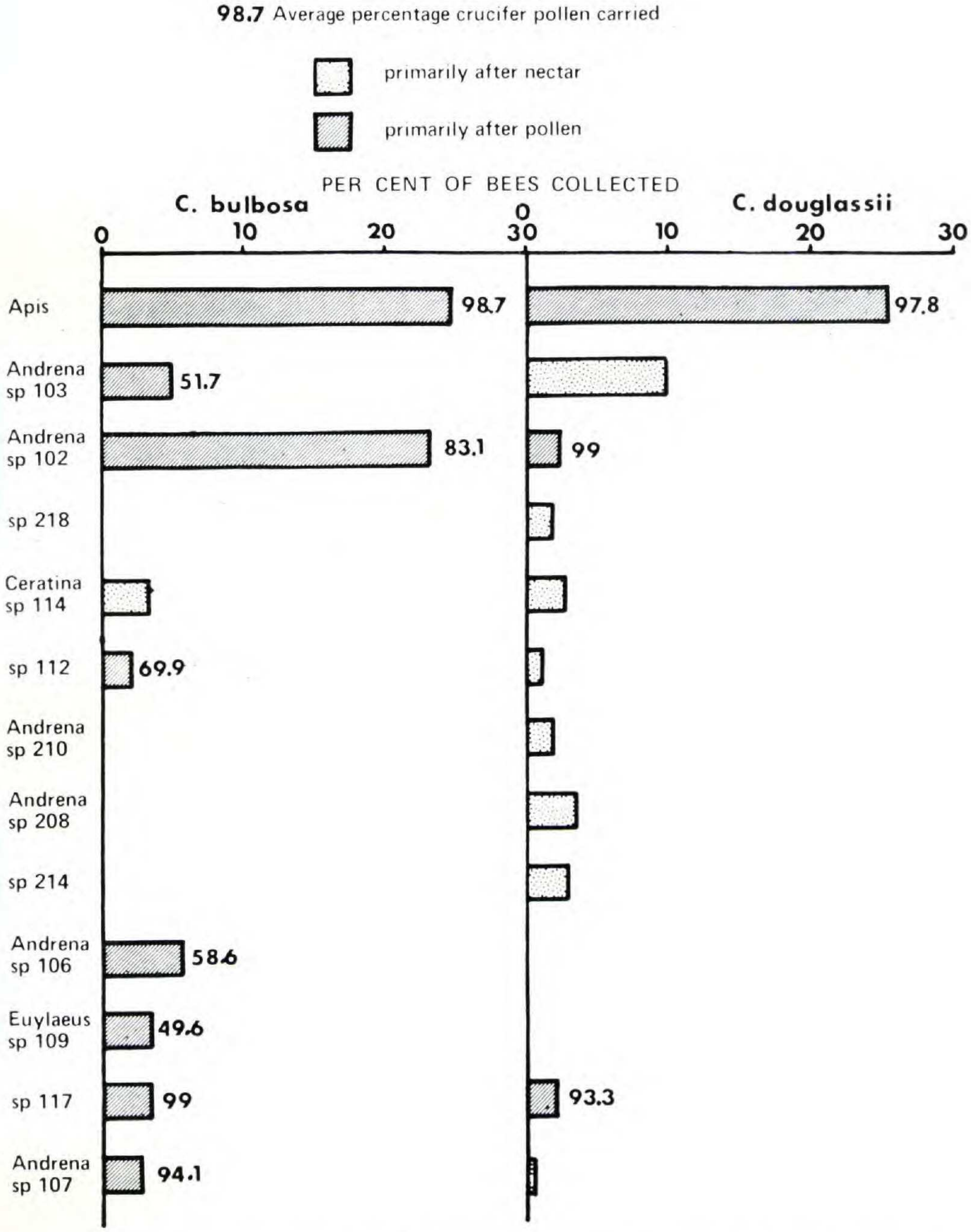


Fig. 15. Visitors to *Cardamine douglassii* and *C. bulbosa* including the percentage of Crucifer pollen carried in their corbicular loads.

used as an indication of the constancy that the bee exhibited for Cruciferae and possibly for the particular crucifer on which it was collected. Bees were identified to genus by using Mitchell's (1960, 1962) keys. Species identification was not attempted in most cases. The specimens have been sent to Dr. R. Fisher at Michigan State University for further verification.

One hundred and forty-three insects were taken visiting *Cardamine bulbosa* and two hundred and fifty-nine visiting *C. douglassii.* The most common bee collected on both species was *Apis mellifera* (Fig. 15). Robertson (1928) did not report honey bees as visitors of *C. bulbosa.* Although honey bees are apparently lacking from many *C. bulbosa* communities, we found that they are active visitors in some. One member of the genus *Andrena* (sp. 102) is very common on *C. bulbosa.* This species, while sometimes found on *C. douglassii,* is of secondary importance on this earlier flowering species.

Apis mellifera is by far the most common visitor of *Cardamine douglassii* (Fig. 16). Another species of *Andrena* (sp. 103) makes about ten percent of the visits to *Cardamine douglassii* and is primarily a nectar feeder. This species is probably not as important a pollinator as its numbers indicate since its method of collecting nectar frequently entails getting it from the outside of the flower (Fig. 17). Near the end of the flowering season of *C. douglassii, Andrena* (sp. 103) began to collect some pollen. This species, therefore, is of more importance later in the season as a pollinator of both species and possibly between the two species.

Apis mellifera is quite constant as to the pollen it collects (Fig. 15). The smaller bees are noticeably less specific as to the plants they visit. This has been reported numerous times before (Grant, 1950). It was further noticed that with the exception of *Apis mellifera,* which collects both nectar and pollen, the visitors to *C. douglassii* are most often after nectar while those of *C. bulbosa* are pollen gatherers.

Figs. 16-17. Fig. 16. *Apis mellifera* foraging on *Cardamine douglassii*. Fig. 17. *Andrena* sp. (sp 103) visiting *C. douglassii*.

The insect species most likely to visit the flowers of both species as far as numbers are concerned appears to be *Apis mellifera*. However, it seems quite species-specific at any one time. This, of course, may not be true with the new bees fresh from the hive (Grant, 1950; Butler, 1945). The two common species of *Andrena* (nos. 102, 103) appear to be less constant as to the flowers they visit and therefore more likely to act as cross pollinators of the two species.

ECOLOGY

Stuckey (1962) and others previously proposed ecological factors as possible isolating mechanisms between the two taxa under investigation. To test this hypothesis twenty-three communities in which one or both of the *Cardamine* species were found (Table 1, 6) were studied. Three transects six feet wide and a minimum of 300 feet long were made through each community in which the species were growing. All of the woody plant species present within the transects were recorded, including their diameter at breast height (DBH). Seedling trees were considered to have a DBH of less than one inch, while sapling trees had a DBH of 1-6 inches and canopy trees had a DBH greater than six inches. This method allowed the calculation of abundance and basal area but did not allow calculation of frequency.

Estimates of species composition and abundance of herbaceous flora within a community were made by sampling five or more one meter square quadrats at random. After visual estimates of the abundance of the various species had been made, they were classified into three categories: abundant (3), common (2), rare (1). Plants present in the community but not located within one of the five quadrats were also noted. Community abundance of the herbaceous species was projected by summing the abundant (3), common (2), or rare (1) values of each species for the five quadrats. In this system a species that was abundant in all five herbaceous quadrats would then have an abundance value of 15.

Soil moisture was calculated at intervals throughout the spring and early summer for two years in many of the communities. Soil from the upper four inches was collected into airtight jars, and weighed immediately upon return to the lab. This soil was dried at 105°C for 48 hours and reweighed. The percent water in the soil was then calculated with percentages greater than 100 percent, assumed to be 100 percent.

Importance percentages of the more abundant woody species were calculated by the Jackson and Petty (1971) method. Jackson and Petty argue that weighing the percent basal area (dominance index) twice as much as the density (percent abundance) gives a more ecologically accurate expression of a species' contribution to a community in disturbed old-growth forests.

The importance value, percent water, abundance, basal area, number of canopy trees per acre, and pH were used in a regression with 17 morphological characters to study character relationships (Hart, 1972; Appendix 8).

Similarities between the study areas were tested with the use of a Cluster Program by G. F. Bonham-Carter (1967). This technique used presence-absence data as opposed to Hall's (1970) semiquantitative data method. Jaccard's coefficient (Sokal & Sneath, 1963; Sneath & Sokal, 1973) was also found to be quite useful. This coefficient prevents a high degree of association from occurring between objects lacking a large number of characters in common. Similarity, as expressed by this coefficient, is a measure of the similarity of plants present in the various communities.

The weighted pair group method, using average linkage, was used in clustering the Operational Taxonomic Units (OTU's) of the association coefficient matrix (Sokal & Sneath, 1963; Sneath & Sokal, 1973).

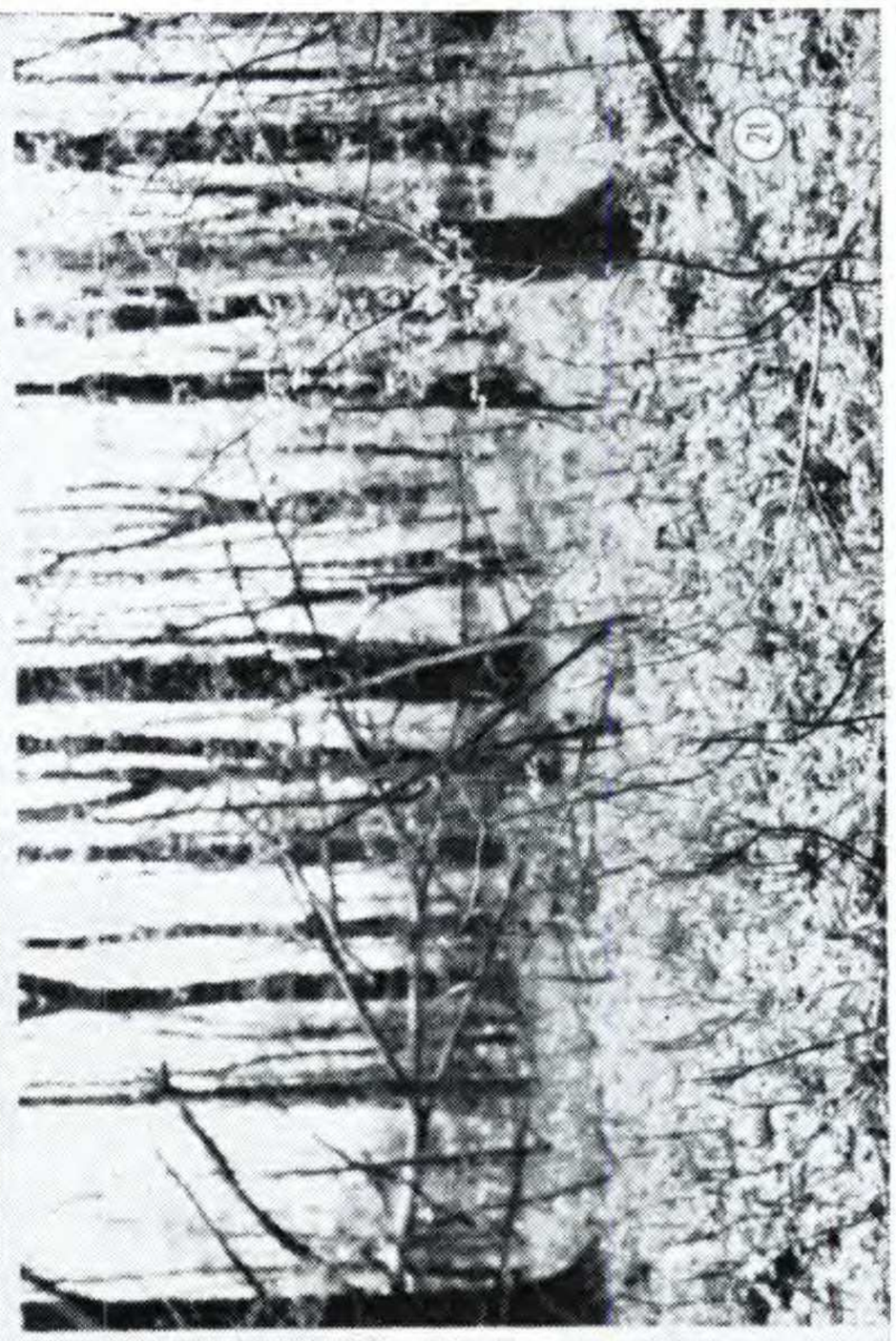
Both the Q-mode or comparison between samples (populations) and the R-mode or comparison between variables (plant species) analyses were used for clustering in this study. The Q-mode association comparing populations is

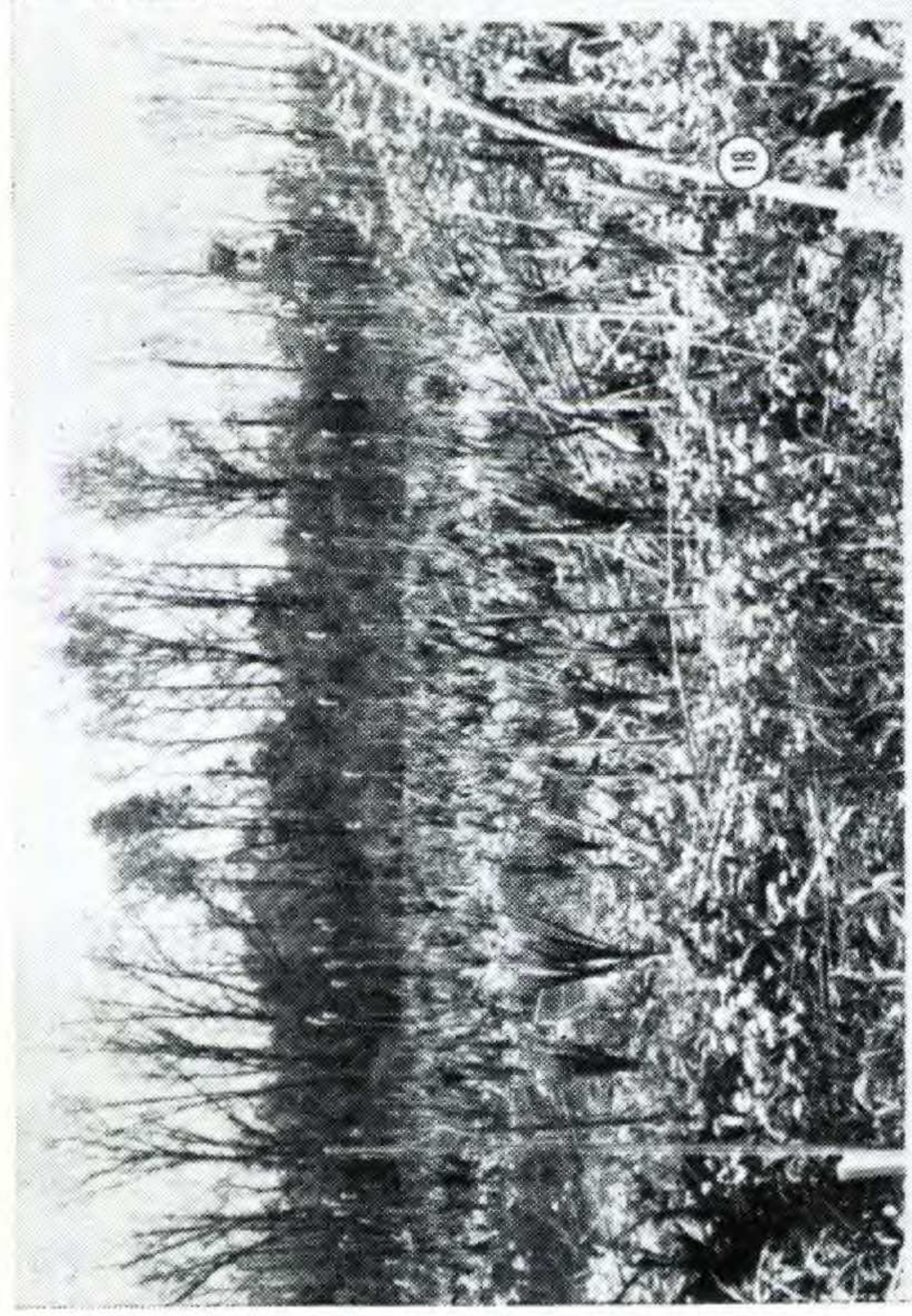
based on presence and absence data while the R-mode analysis is based on semiquantitative data.

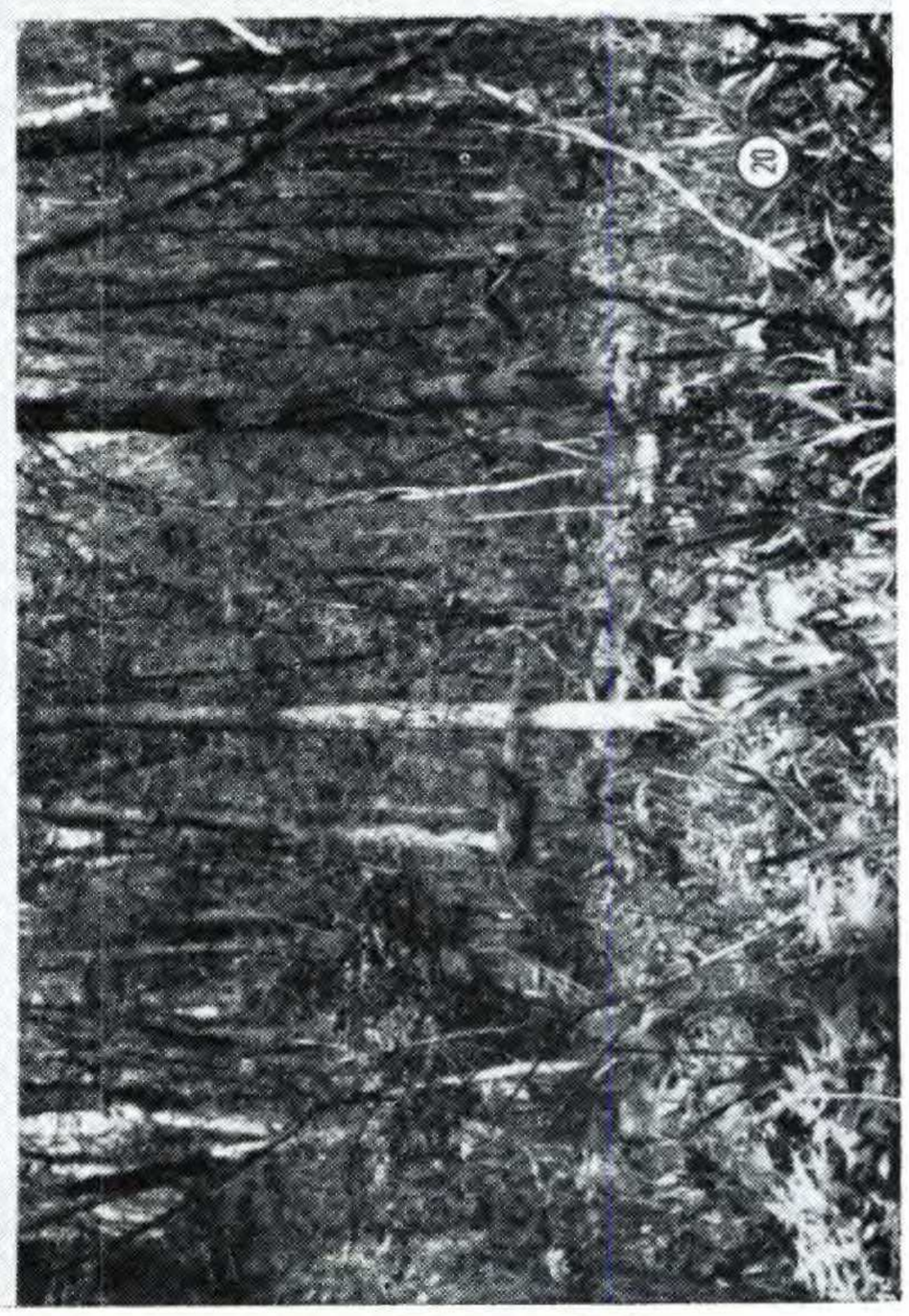
Twenty-three populations containing *Cardamine douglassii* and/or *C. bulbosa* were compared for their herbaceous vegetation. Both Jaccard's coefficient and Sokal and Michner's (Sokal & Sneath, 1963; Sneath & Sokal, 1973) coefficient were used. Of a total of 191 herbaceous species encountered within one or more of the twenty-three populations, only those ninety-seven species present in two or more communities were used. This arbitrary limitation was necessary due to the lack of a computer program capable of handling a matrix including all the species.

Community similarity, based on the presence or absence of all sixty-eight woody species encountered, was computed by both Jaccard's and Sokal and Michner's association coefficient and clustered by both the weighted and unweighted method. Only twenty-two populations were compared as opposed to the twenty-three populations used in comparison of the herbaceous species. This was necessary since there are no woody species in one community studied near Cincinnati, Ohio, which is periodically mowed throughout the summer and fall.

The R-mode approach, which is based on only presence or absence of a species, has been questioned as a statistic by Siegal (1956). For this reason the species comparison has been based on semiquantitative data (Hall, 1970). The community abundance of each species ranges from one through fifteen. This abundance was split into five groups: abundant (11-15), very common (6-10), common (2-5), rare (from present in community but not in a quadrat to a community abundance of one), and absent. This particular breakdown of community abundance values was chosen since it allowed each of the categories to contain a more equal number of species. It was also felt that this would give increased cognizance of the rarer species while decreasing the relative importance of the more common and abundant species.







Figs. 18-21. Fig. 18. *Cardamine bulbosa* community, Spring Valley, Ohio. Fig. 19. *Cardamine bulbosa* community, Cincinnati Country Day School, near Cincinnati, Ohio. Fig. 20. *Cardamine douglassii* community, Bachelor Estate, near Oxford, Ohio. Fig. 21. *Cardamine douglassii* and *C. bulbosa* community, many putative hybrids present, near Bowling Green, Ohio.

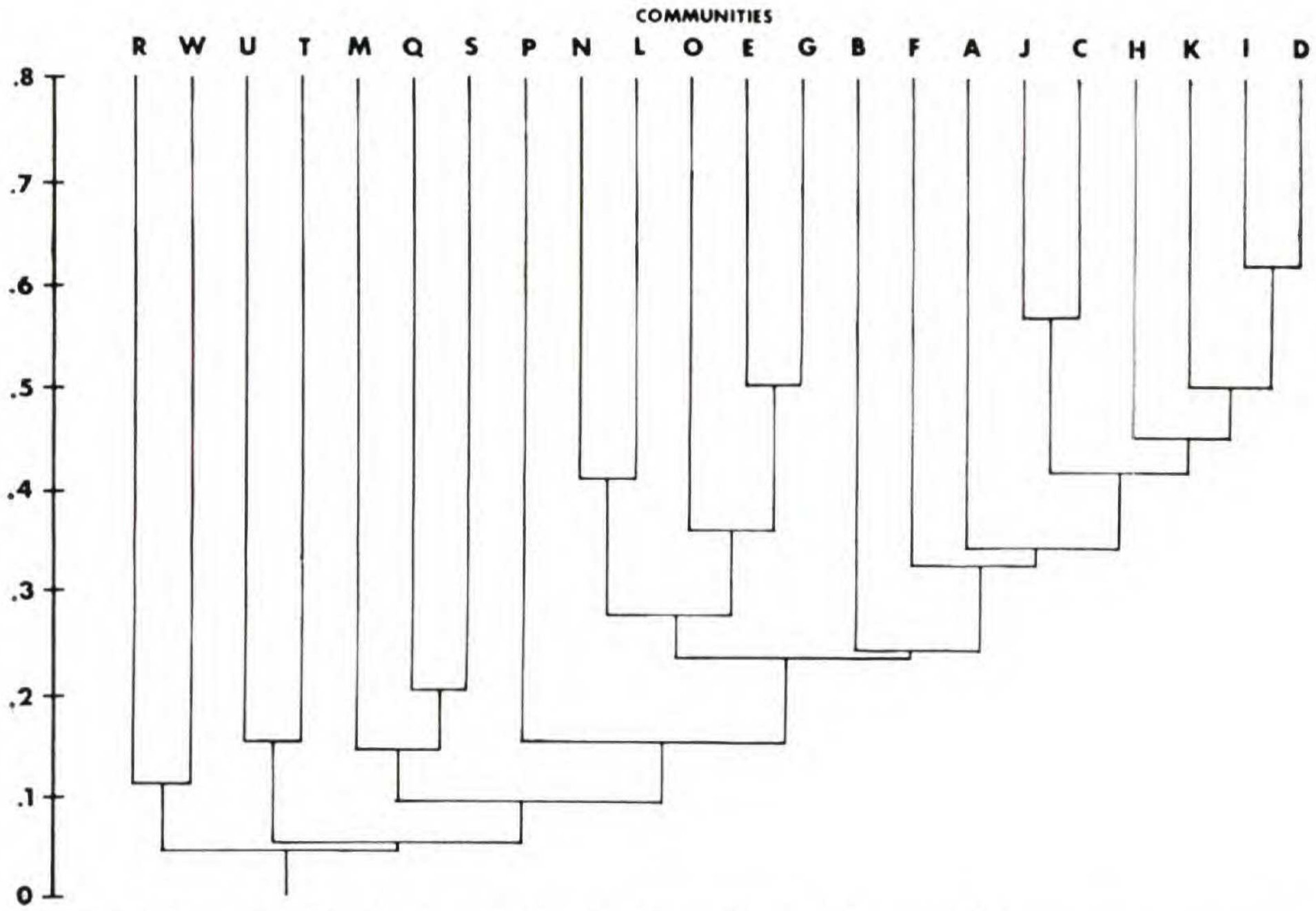


Fig. 22. Population similarity based on the woody species present in the community. Jaccard's coefficient clustered by the weighted pair-group method. Mean expected value = 0.0932.

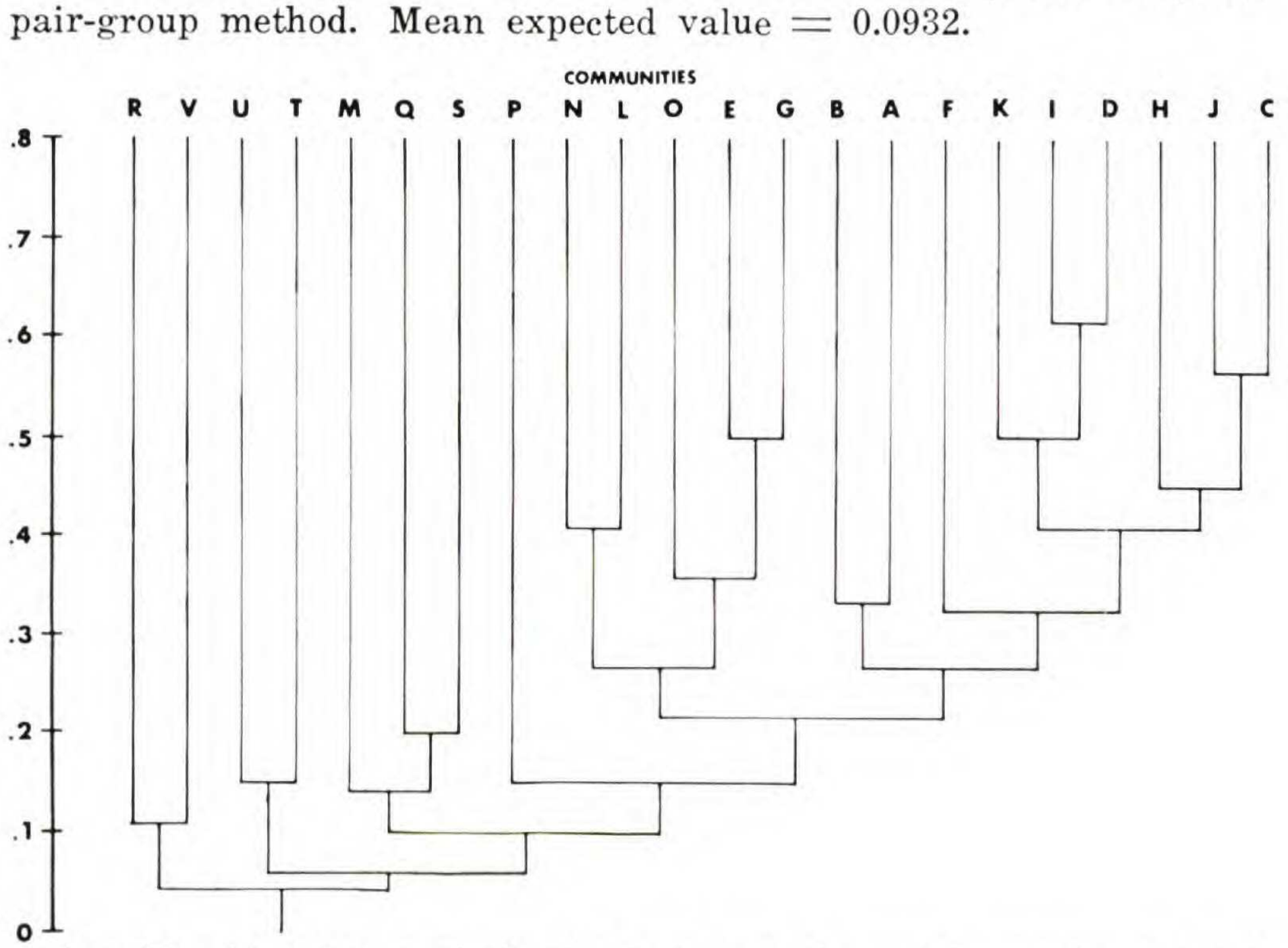


Fig. 23. Population similarity based on the woody species present in the community. Jaccard's coefficient clustered by the unweighted pair-group method. Mean expected value = 0.0932.

The variation of habitats in which the species were found is shown in the four community photographs (Figs. 18-21). Similarity based on the presence of woody species (Jaccard's coefficient) does not reveal a clear cut separation (Fig. 22, 23). However, several interesting associations do occur. The community at Rush Run (Com. G) containing *Cardamine douglassii* is different from the others in southwestern Ohio. It has a strong association (about 0.5) with the northern community in Bedford, Ohio (Com. E). Both communities have relatively high amounts of water in the soil. Both of these communities cluster with three *C. bulbosa* communities, one from the Illinoian till plain of southwestern Ohio (Com. O) and two others from Kentucky (Com. N, L). It is also of interest to note that the woody species on the hillside and the adjacent floodplain at the John Bachelor Preserve (Com. I, J) near Oxford, Ohio, are not very similar as they fail to cluster closely together.

The Sokal and Michner coefficient takes into consideration the absence of a species and causes some noticeable changes in the form of the dendrogram (Fig. 24, 25). For instance, Cedar Swamp, an alkaline bog with northern affinities (Dachnowski, 1910), is shown to be unlike all other communities. The weighted cluster (Fig. 24) shows five major groups. One is the community at Cedar Swamp (Com. T). The second is the two communities studied near Bowling Green, Ohio (Com. B, A), which have both *Cardamine* species and apparent hybrids intermixed. The third group contains a mixture of communities: one *C. douglassii* from southwestern Ohio (Com. G), one *C. douglassii* from northern Ohio (Com. E), one from the Illinoian till plain of southern Ohio (Com. O), and two from Kentucky containing *C. bulbosa* (Com. N, L). The fourth group contains the six remaining communities in which *C. douglassii* (Com. C, D, H, I, J, K) is important. The remaining cluster contains communities which have relatively large numbers of *C. bulbosa,* although one (Com. P) does contain a few *C. douglassii.*

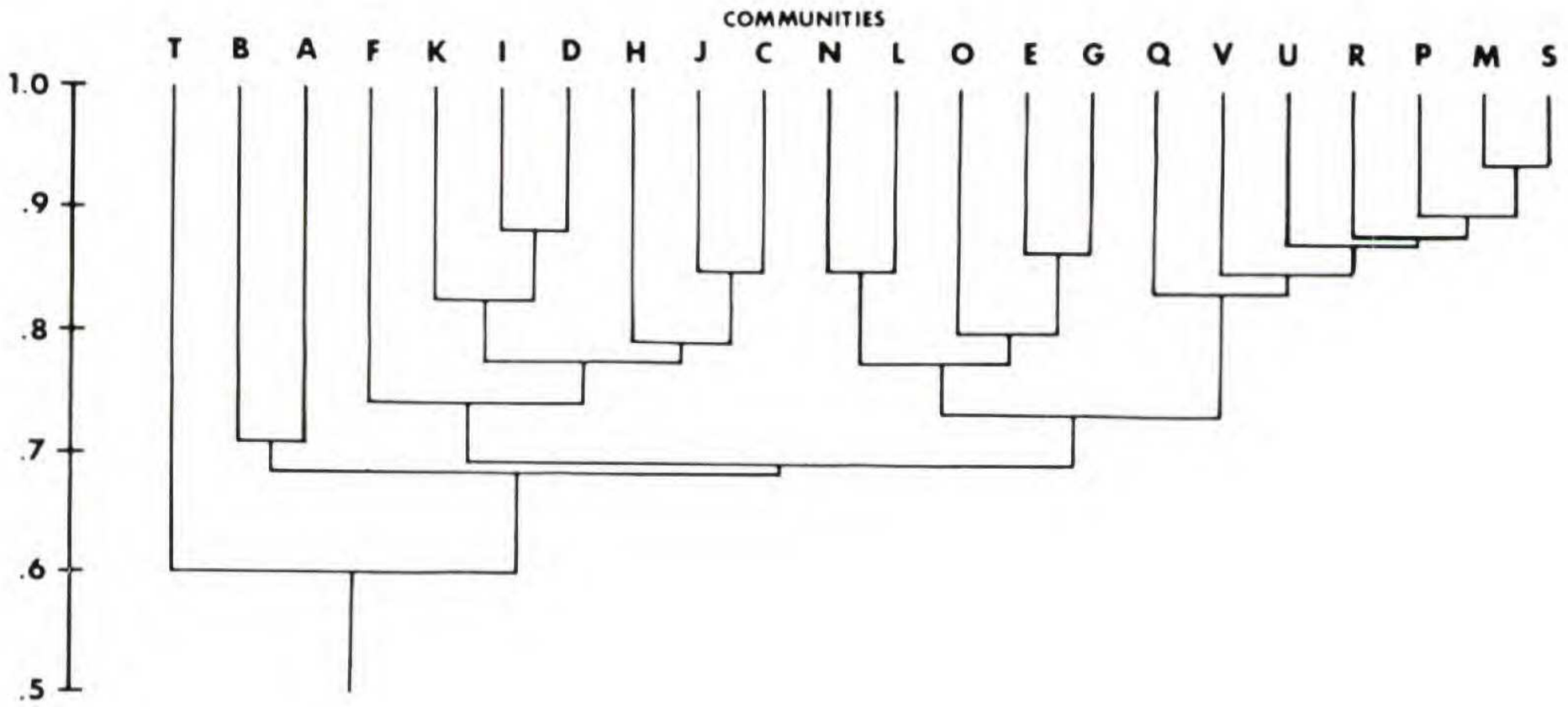


Fig. 24. Population similarity based on the woody species present in the community. Sokal and Michener's coefficient clustered by the weighted pair-group method. Mean expected value = 0.694.

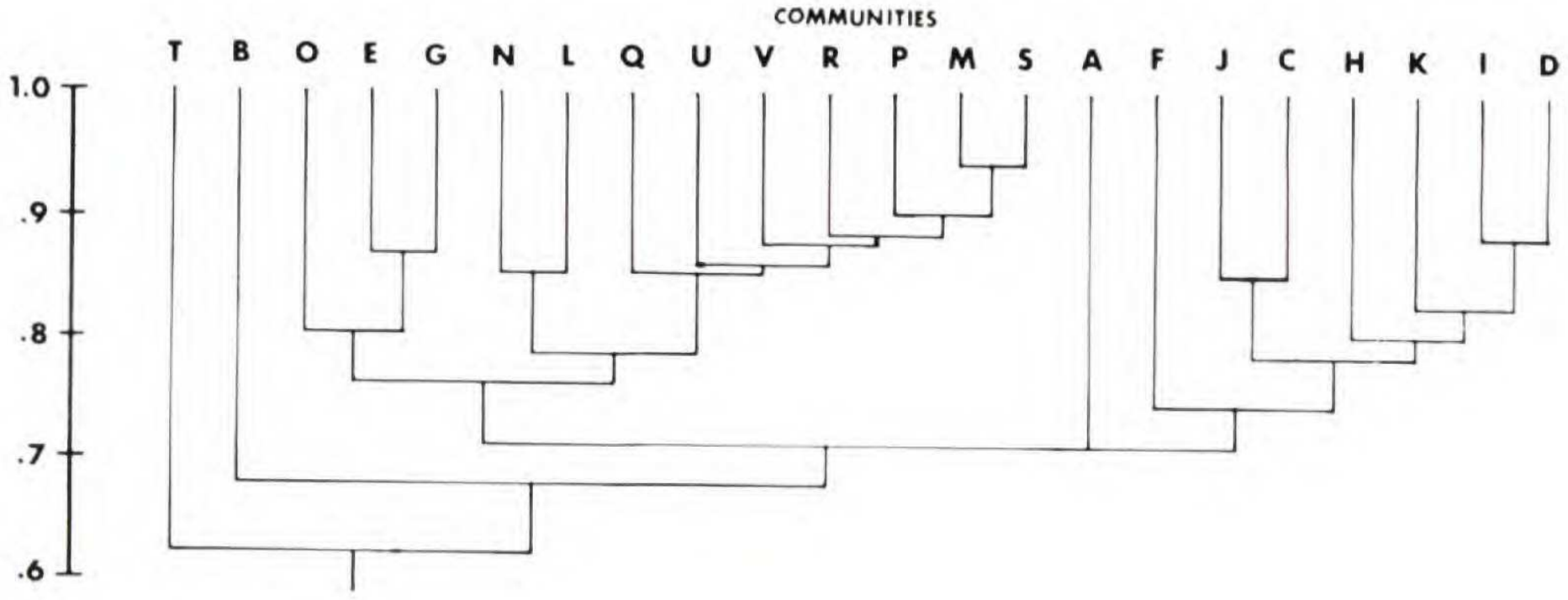


Fig. 25. Population similarity based on the woody species present in the community. Sokal and Michener's coefficient clustered by the unweighted pair-group method. Mean expected value = 0.0694.

The unweighted pair group method (Fig. 25) is basically similar but with the Kentucky communities (Com. L, M, N) and two communities containing *Cardamine douglassii* (Com. E, G) clustering with the communities containing large numbers of *C. bulbosa*. The Cedar Swamp community (Com. T) and one from Bowling Green (Com. B) are somewhat isolated from the others.

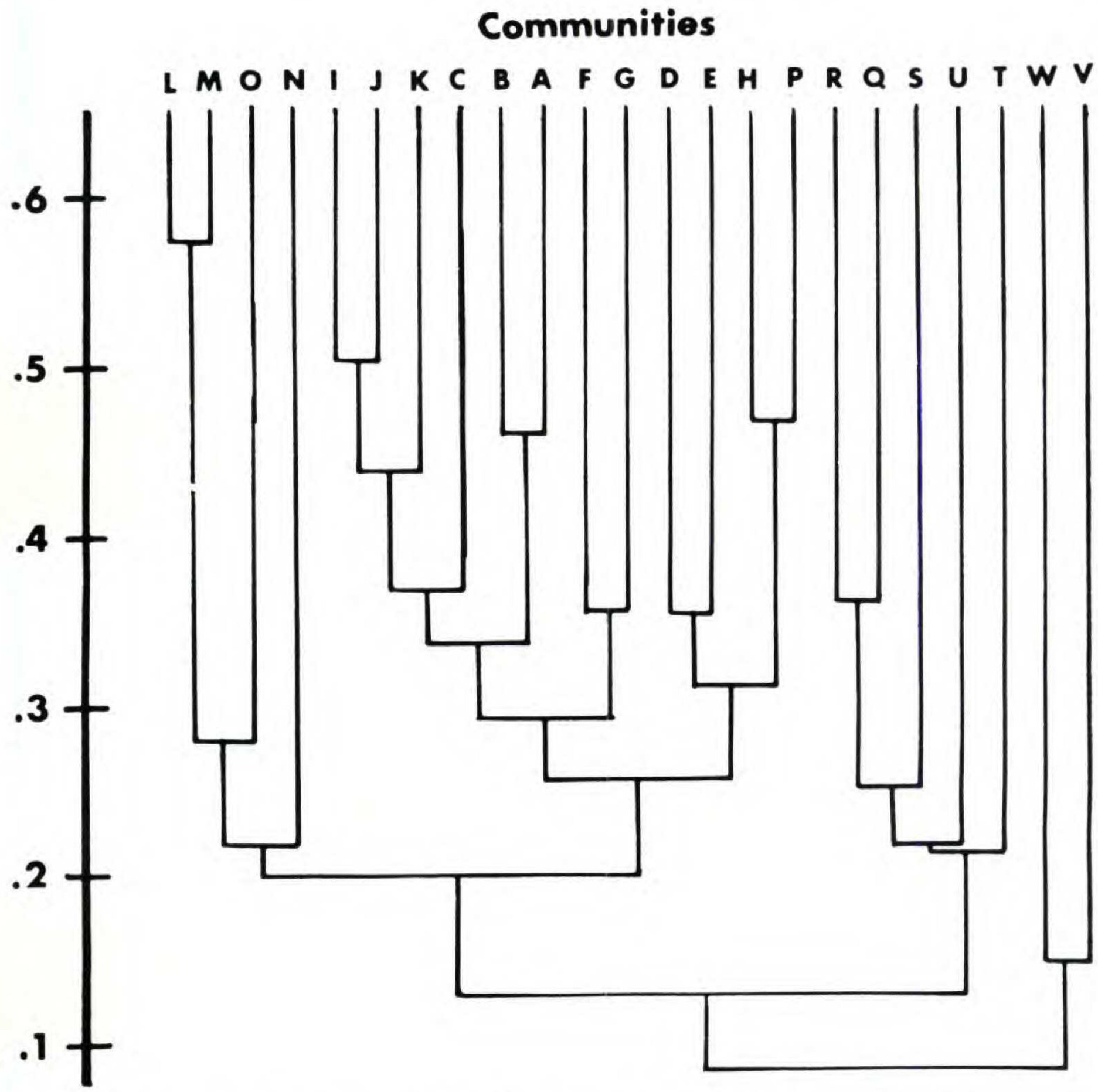


Fig. 26. Population similarity based on the herbaceous species present in the community. Jaccard's coefficient clustered by the weighted pair-group method. Mean expected value = 0.105.

Jaccard's coefficient based on the presence of herbaceous species produces four easily recognizable clusters (Fig. 26, 27). One group (Com. L, M, O, N) contains the communities from the Illinoian till plain east of Cincinnati, Ohio, and from the unglaciated populations of Kentucky. Others containing *Cardamine bulbosa* but not *C. douglassii* (Com. R, Q, S, U, T, V) are high in water except for the unusual community (Com. W) where *C. bulbosa* is found in a mowed field (Table 6). The remaining group is composed of communities where *C. douglassii,* with or without *C. bulbosa,* is found.

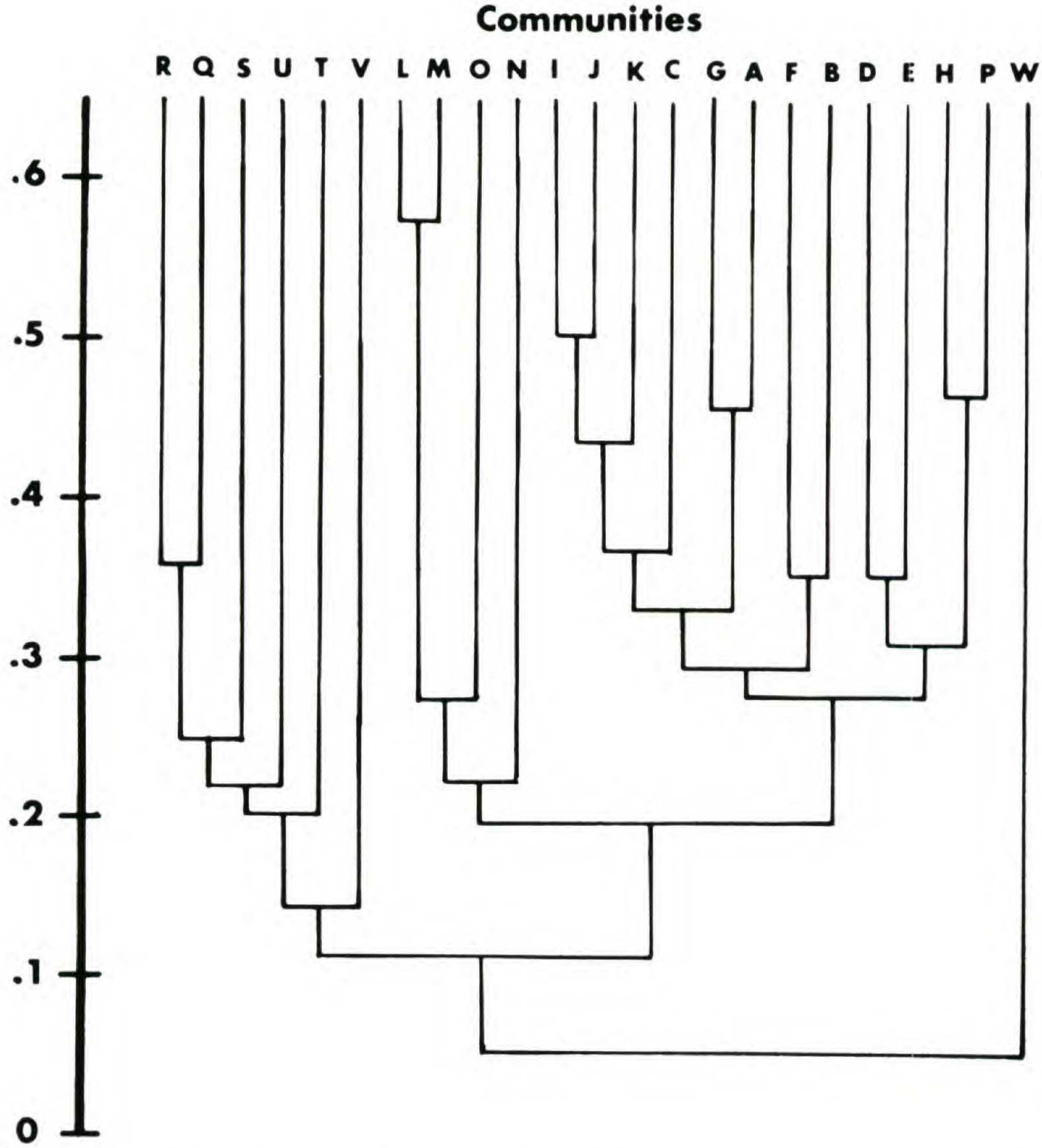


Fig. 27. Population similarity based on the herbaceous species present in the community. Jaccard's coefficient clustered by the unweighted pair-group method. Mean expected value = 0.105.

When considering both presence and absence (Sokal & Michner's coefficient), the unglaciated populations do not form a separate cluster. The weighted pair-group method (Fig. 28) separates the twenty-three communities into two major groups. This division is not related to particular *Cardamine* species but appears to be associated with the number of large woody plants present and the presence of large amounts of water. The communities in the smaller

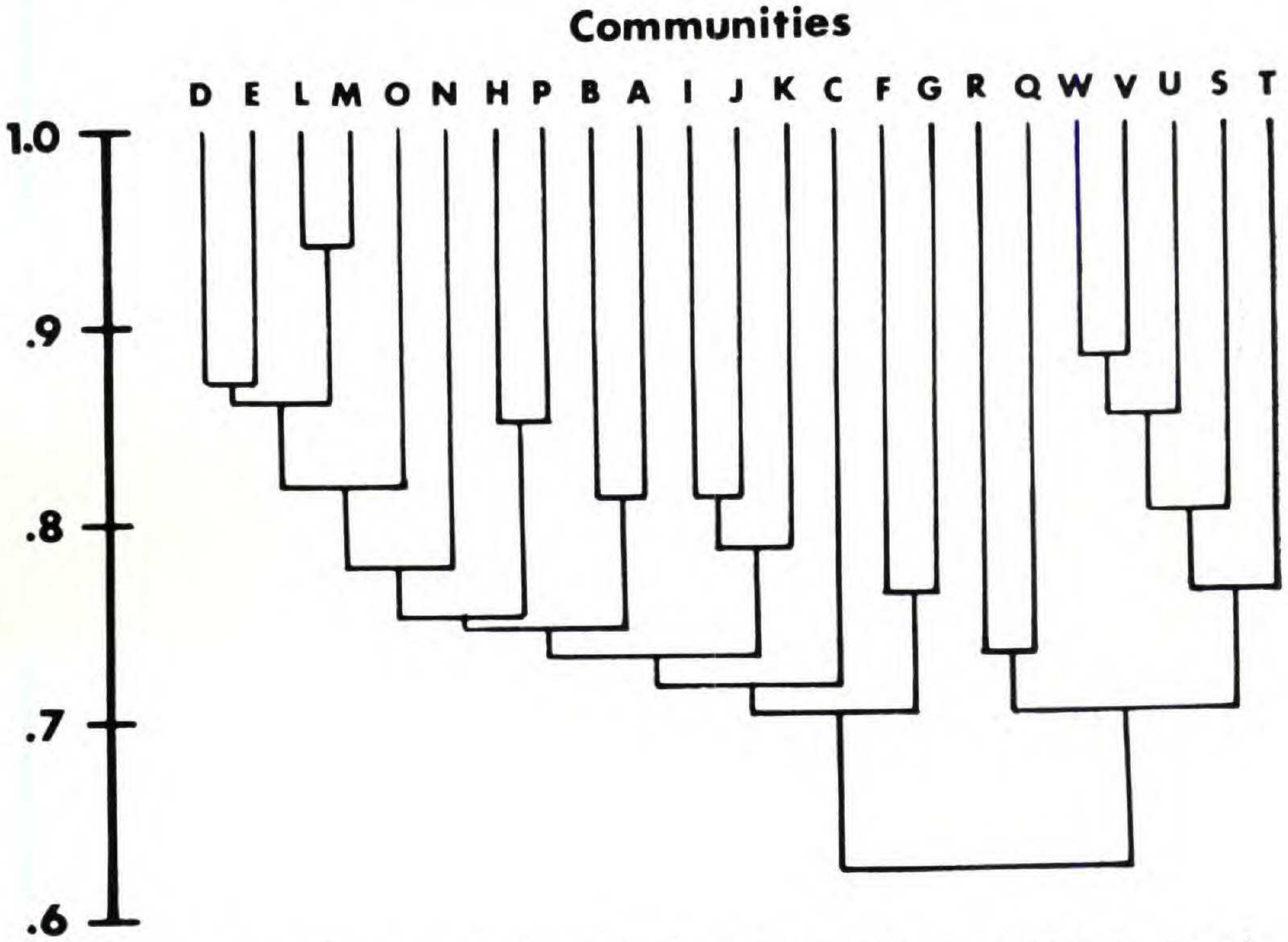


Fig. 28. Population similarity based on the herbaceous species present in the community. Sokal and Michener's coefficient clustered by the weighted pair-group method. Mean expected value = 0.8204.

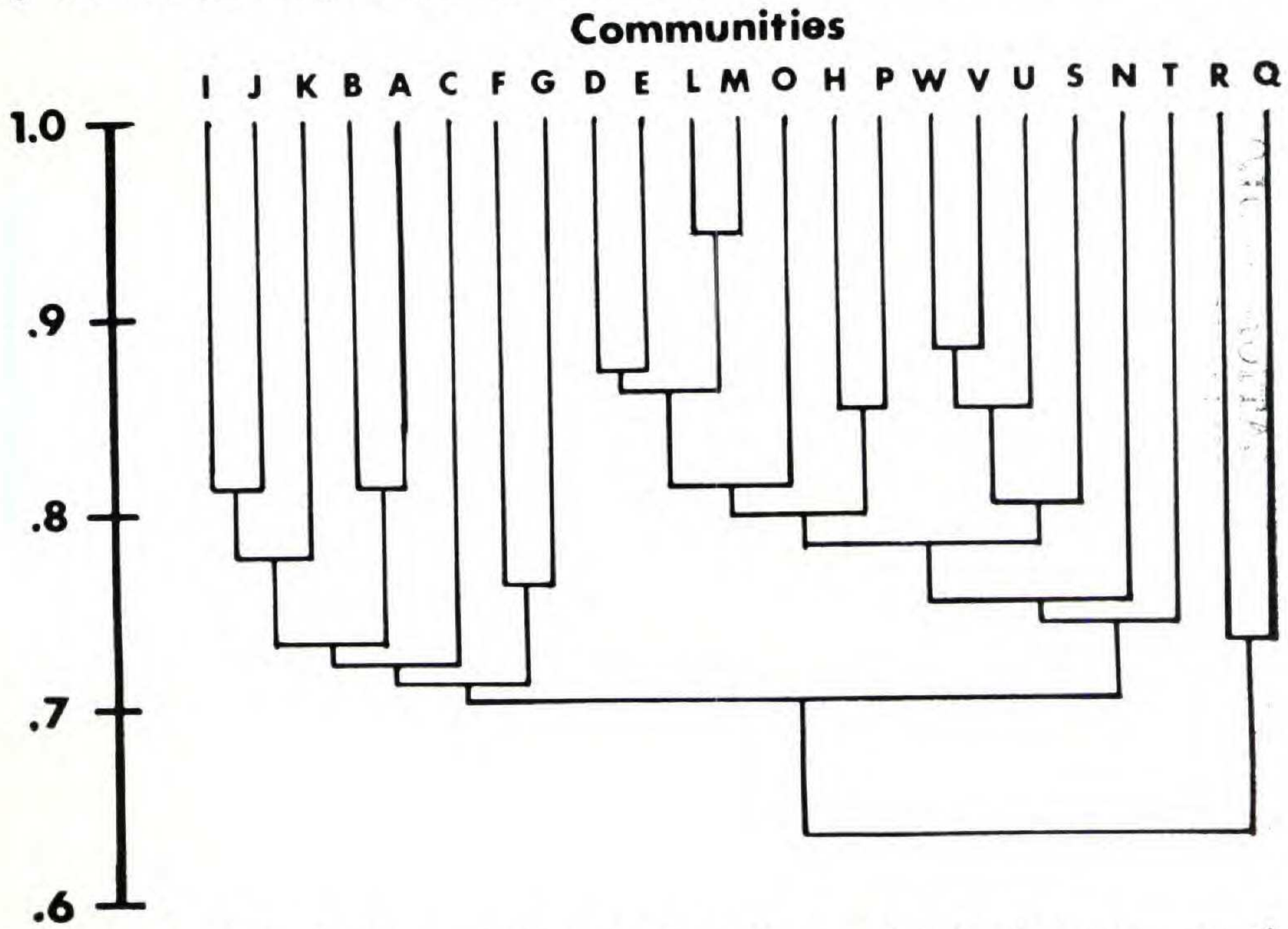


Fig. 29. Population similarity based on the herbaceous species present in the community. Sokal and Michener's coefficent clustered by the unweighted pair-group method. Mean expected value = 0.8204.

TABLE 6. POPULATION ECOLOGY OF TWENTY-THREE POPULATIONS OF *CARDAMINE BULBOSA* AND/OR *C. DOUGLASSII*

Population	Species*	Total Abundance Per Acre (Ab.)	Total Basal Area in Sq. In. Per Acre (BA)	Importance Value (BA+BA+Ab) ÷3	Abundance Percent Seedling	Abundance Percent Sapling	Abundance Percent Canopy	Average Ph	Percent Water
A	*D*B	2346	20,599	14,515	43.75	34.7	5.6	6.99	100
B	*D*B	1082	18,990	13,021	52	24	24	7.08	95
C	*D*B	1201	13,336	9,291	81	13	5	7.08	87
D	D	2673	47,534	32,580	44	26	11	6.36	63
E	D	1424	18,524	12,824	51	30	17	5.8	84
F	D	1601	38,417	26,145	51	41	7	6.47	57
G	D	1645	20,287	14,073	59.0	34.8	6.2	5.9	71.86
H	DB	1389	4,724	3,612	50	49	1	8.15	60
I	D	1144	17,892	12,309	46	46	8	7.3	38
J	*D*B	1092	12,145	8,461	51	37	11	8.6	33
K	D	2142	25,578	17,766	66	22	11	6.45	51
L	B	1109	19,948	13,668	70.4	18.5	11.1	—	100
M	B	369	21,818	14,668	33	22	44	—	100
N	B	1591	16,433	11,486	73.25	22.1	4.65	—	46.4
O	B	1085	44,411	29,969	68	18.7	13.3	6.1	100
P	*B*D	630	7,922	5,491	75.7	13.5	10.8	5.73	100
Q	B	1498	8,154	5,935	72.8	20.3	6.7	7.3	67
R	B	26	46.5	40	0	100	0	7.5	100
S	B	92	2,531	1,718	80	20	0	7.24	100

T	B	1084	28,337	19,253	54.5	40.9	4.5	6.94	100
U	B	10,418	14,900	13,406	92.5	7.2	0.6	5.96	100
V	B	1642	3,088	2,606	83	17	0	—	100
W	B		mowed field					5.7	

*B = *C. bulbosa*
D = *C. douglassii*
Italics indicate that the species is most abundant in that community

group tend to be more open with nearly 100 percent water present whereas the larger group has large trees and tends to be drier. Three groups may be distinguished by the unweighted pair-group method (Fig. 29). While these groups are based on the presence and absence of various species, there is no apparent ecological factor associated with the separation of the communities in this manner (Table 6).

Herbaceous species associated with *Cardamine bulbosa* (Jaccard's coefficient) appear to be different from those associated with *C. douglassii*. The ten highest association values of species found with *C. bulbosa* and *C. douglassii* are different for the herbaceous species, although the almost ubiquitous *Rhus radicans* and *Parthenocissus quin-*

TABLE 7. HERBACEOUS SPECIES WITH THE HIGHEST ASSOCIATION (JACCARD'S) WITH *CARDAMINE BULBOSA* AND *C. DOUGLASSII*[1]

Species	Association
Association with *Cardamine bulbosa*	
Galium aparine	.345
Parthenocissus quinquefolia	.340
Impatiens biflora	.338
Symplocarpus foetidus	.316
Rhus radicans	.294
Eupatorium perfoliatum	.243
Urtica dioica	.220
Caltha palustris	.205
Angelica atropurpurea	.195
Scirpus atrovirens	.189
Association with *Cardamine douglassii*	
Geum vernum	.593
Osmorhiza claytoni	.583
Galium aparine	.564
Viola papilionacea	.556
Cryptotaenia canadensis	.500
Parthenocissus quinquefolia	.487
Rhus radicans	.472
Amphicarpa bracteata	.444
Claytonia virginica	.407
Aster sp.	.360

[1]*C. bulbosa* with *C. douglassii* = .130

quefolia are found with both species (Table 7). When determining which species are most characteristically associated with any one particular species habitat, one cannot consider the absence of a species from an area as being as important as its presence. For this reason it is thought that Jaccard's coefficient best describes the species associated or characteristically found with either *C. bulbosa* or *C. douglassii.*

Jaccard's coefficient (Table 7) shows only a few species highly associated with both *Cardamine douglassii* and *C. bulbosa.* This would be expected if the two species differed in ecological requirements.

Table 8 shows the ten herbaceous species which have the greatest difference between the association with *Cardamine bulbosa* and the association with *C. douglassii.* These may be considered as differential indicators to be used in identifying the species of *Cardamine* expected to be found within a given area. However, one should not assume that all of these species must be present with either *C. bulbosa* or *C. douglassii.*

The importance percentages (Table 9) of the woody species fail to show any clear cut habitat difference between populations of *Cardamine bulbosa* and *C. douglassii.* Close examination, however, does show a dissimilarity of the *C. bulbosa* communities on and off Wisconsin glaciation zones. *Acer saccharum* and *Ulmus americana* are more characteristic of *C. douglassii* and unglaciated *C. bulbosa* communities.

Analysis of both the woody and herbaceous vegetation fails to completely separate populations containing *Cardamine bulbosa* from those containing *C. douglassii.* The R-mode analysis of the herbaceous vegetation does indicate particular plant species commonly associated with one species but not the other. This is an indication of some ecological differences between *C. bulbosa* and *C. douglassii.* That this isolation mechanism is not complete may be seen by the communities in which both *Cardamine* species are found.

TABLE 8. HERBACEOUS SPECIES WITH THE MOST DIFFERENCE BETWEEN THEIR ASSOCIATION (JACCARD'S) WITH *CARDAMINE BULBOSA* AND THAT OF *C. DOUGLASSII*

Species	*Association values* D	B	Difference
Association with *Cardamine bulbosa*			
Symplocarpus foetidus	.121	.316	.195
Scirpus atrovirens	0.0	.189	.189
Carex stipitata	0.0	.179	.179
Sagittaria latifolia	0.0	.162	.162
Typha latifolia	0.0	.162	.162
Carex laxiflora	0.0	.154	.154
Boehmeria cylindrica	0.0	.135	.135
Caltha palustris	.063	.205	.142
Onoclea sensibilis	.032	.154	.122
Senecio aureus	.067	.184	.117
Eupatorium perfoliatum	.138	.243	.105
Association with *Cardamine douglassii*			
Geum vernum	.593	.143	.450
Osmorhiza claytoni	.583	.133	.450
Viola papilionacea	.556	.146	.410
Cryptotaenia canadensis	.500	.156	.344
Amphicarpa bracteata	.444	.130	.314
Hystrix patula	.333	.023	.310
Campanula americana	.320	.022	.298
Aster sp.	.360	.068	.292
Sanguinaria canadensis	.333	.071	.262
Polymnia canadensis	.250	0.0	.250
Claytonia virginica	.407	.159	.248

In general there is separation, as indicated in the dendrograms, between the populations of *Cardamine bulbosa* found in swampy conditions and those of Wisconsin glaciation. Populations of *C. douglassii* with or without *C. bulbosa* seem to cluster together.

The analysis of the woody and herbaceous vegetation, which shows a tendency of *Cardamine douglassii* containing communities to cluster together, is a strong indication of the specificity of *C. douglassii* for a particular habitat. *Cardamine bulbosa,* on the other hand, appears to be quite

variable as to habitat, with a much larger range of habitat conditions under which it is capable of undergoing ecesis.

Herbaceous species are found to be associated with *Cardamine bulbosa* at a lower level than with *C. douglassii.* This fact may also be interpreted to mean that the particular study areas containing *C. bulbosa* varied more than those containing *C. douglassii* and that the resulting associations are due to sampling error or the fact that the ecology of *C. bulbosa* is more variable than that of *C. douglassii.*

MORPHOLOGY

To be able to more fully understand the evolutionary relationships between and within *Cardamine douglassii* and *C. bulbosa,* it was considered necessary to take a closer look at the morphological similarities and differences within the taxa. Plants for morphological study were selected at random from within each population. Twenty-two characters were measured on each plant. Seven of these characters were averages, such as the length of the pubescence, length of petals, etc. Through the use of various indices the number of characters was increased to thirty-one (Table 10).

Three measurements were made to the nearest millimeter (Character Numbers 2, 3, 4) and three (number of branches, number of leaves, number of hairs per mm^2) were meristic in nature. Length of pubescence and number of hairs per square millimeter were calculated using an ocular micrometer. Other measurements were made with calipers to the nearest .001 mm.

When the plants were collected in the field, one or two flowers were picked. Since petal size may vary depending on the age of the flower, the flowers used were all of about the same age. A flower was dissected, the parts placed on ozalid paper and then exposed to light. Later exposure to ammonium hydroxide caused the shaded portion that had been covered by the flower parts to become dark. Measurements of the images were made with a Bausch and Lomb measuring magnifier to the nearest tenth of a millimeter.

TABLE 9. IMPORTANCE PERCENTAGES (GREATER THAN 5%) OF WOODY SPECIES IN THE TWENTY-THREE COMMUNITIES

Community Letters	R	V	U	T	M	Q	S	P	N	L	O	E	G	B	A	F	K	I	D	H	J	C
Population Number	8	11	3	9		7		4	10	1	6	15	17		12,13	16	20		14	5,18	19	14
Species:																						
Salix nigra	100					42.7	53.1															
Fraxinus americana			91.8	10.6	31.7		6.9	34.7	8.9	12.7		10.2					17.5	59.1		7.0	29.8	
Juglans nigra					7.1											8.0					19.1	
Acer negundo				15.4		36.5									22.7	9.4				17.2		
Cornus amomum	68.9			22.6																		
Quercus macrocarpa				6.8																		
Thuja occidentalis		22.3																				
Lindera benzoin		6.5				28.3	14.3		7.4													
Fraxinus pennsylvanica		29.1				26.1									36.0							
Juglans cinerea		36.3																				
Platanus occidentalis							8.4															37.8
Carpinus caroliniana							13.5								5.2		11.4					

Acer saccharum					44.1			5.4	6.8		8.4			8.4	21.3			
Ulmus americana				8.5	12.8			30.4	7.9							30.1	20.8	
Alnus rugosa		62.4																
Acer rubrum		28.1					64.8											
Rhus vernix		7.9																
Carya ovata							8.1	10.2	27.8									
Quercus alba							22.3					30.4						
Quercus falcata						10.0												
Viburnum dentata	21.5																	
Salix sp.	8.5																	
Acer saccharinum										5.6								
Ostrya virginiana									9.1	9.5			5.3					
Cercis canadensis										16.4			6.0					
Ulmus fulva										16.7		5.5	5.3	56.6				
Liriodendron tulipifera						8.4												
Celtis occidentalis								14.4				7.8						
Quercus bicolor								6.2										
Prunus serotina								9.6										
Carya cordiformis						12.4		34.5										
Corylus americana								8.6										
Quercus borealis				30.5	6.2													
Crataegus sp.				5.9														
Gleditsia triacanthos											26.5							
Cornus florida						6.4												
Sassafras albidum						35.4												

TABLE 10. CHARACTERS USED IN THE MORPHOLOGICAL STUDY

1. Number of branches
2. Height to first pedicel from the base of the stem (cm)[1, 2]
3. Height to first leaf from the base of the stem (cm)
4. Height to second leaf from the base of the stem (cm)[2]
5. Length of petiole of first leaf (cm)
6. Length of lowest cauline leaf (cm)[2]
7. Width of lowest cauline leaf (cm)
8. Length of petiole of second leaf (mm)
9. Length of second leaf (cm)[1, 2]
10. Width of second leaf (cm)
11. Least width of second leaf (cm)
12. Number of cauline leaves[1, 2]
13. Height of plant ÷ number of branches (no. 2 ÷ no. 1)[2]
14. Height of first leaf ÷ petiole height (no. 3 ÷ no. 2)[2]
15. Leaf one: petiole-blade index (no. 5 ÷ no. 6)
16. Leaf one: leaf index (no. 7 ÷ no. 6)[2]
17. Leaf two: petiole-blade index (no. 8 ÷ no. 9)
18. Leaf two: leaf index (no. 10 ÷ no. 9)[1, 2]
19. Height ÷ number of leaves (no. 2 ÷ no. 12)
20. Number of hairs per mm (avg.)[1, 2]
21. Average pubescence length (0.01mm)[1, 2]
22. Width of stem (mm)[2]
23. Length of calyx (avg.) (mm)
24. Length of petals (avg.) (mm)[1, 2]
25. Width of petals (avg.) (mm)[2]
26. Petal index (no. 25 ÷ no. 24)[1, 2]
27. Filament length (avg. of long stamens in mm)[2]
28. Pistil length (mm)[2]
29. Gyno-andro index (no. 27 ÷ no. 28)[1, 2]
30. Leaf two width index (no. 11 ÷ no. 10)[1, 2]
31. Color petals (from 1 to 7)[1]

[1]Characters studied in comparison of intrapopulational differences of *C. bulbosa*.

[2]Characters used in calculating Davidson and Dunn coefficient.

Since this was a study of the biosystematics of *Cardamine bulbosa* and *C. douglassii,* it was felt that the inclusion of hybrids collected in a population could be misleading when comparing the populations morphologically. This was a problem in only one community, near Bowling Green, Ohio, where both species and their putative hybrids occur quite commonly. Hybrids were determined based on their intermediate pubescence length and intermediate flowering time, and plants judged to be hybrids were eliminated from the morphological species comparison. This is, then, an attempt to compare only the so called "pure" species.

The recommended sample size for similar taxonomic treatments is from 15-25 individuals (Cazier & Bacon, 1949). Pimentel (1958) investigated the effect of sample size on the range of confidence limits. He found a decrease in the size of the confidence limits with an increase in sample size. About fifteen samples are required to stabilize these limits. A partially successful attempt was made in this study to collect a minimum of fifteen plants from each population. In three populations of the twenty populations (Table 1) the scarcity of specimens necessitated collections of less than fifteen plants. These areas were Pymatuning Lake, Ohio; Camp Hook, Ohio; and at Pulltight Springs, Missouri (population numbers 11, 14, and 2).

Physiogeographic variability may be a significant factor influencing plant morphology. This indicates the importance of equidistance between sample areas. Frequent sampling in similar nearby habitats may represent wasted time and energy while too infrequent sampling may divide a gradual cline into several distinct taxa. Of necessity, most of the twenty populations studied were in southwestern Ohio with scattered areas in northern Ohio, Kentucky and Missouri. Although this cannot be considered a complete study of both taxa, it is at least respresentative of those plants in the mid-West.

The mean, standard deviation, maximum and minimum were computed for the characters in the twenty populations (Hart, 1972, Appendix 11). Significant differences or similarities between populations were established using an analysis of variance for each particular character (Table 10). For this test, data for the particular character were used for all plants measured with a maximum of thirty plants per population.

When the F value was significant ($0.01 \leq p \leq 0.05$) or highly significant ($p < 0.1$) the Student Newman Keuls (SNK) Multiple Range Test (Woolf, 1968) was used to compare the means and show where the similarities and differences were located. The probability level of rejecting the null hypothesis was 0.05.

Petal color was studied by comparing specimens with other specimens instead of with a color chart (Kerlan, 1965). Three different colored petals from three different plants were chosen as standards. One petal was very light pink, another pinkish-purple and the third, rather dark purple. Any specimen lighter than light pink (2) was considered white (1), while those darker than purple (6), were reported as dark purple (7). The measurement of petal color necessitated the use of non-parametric methods which are used when one is concerned with the distribution of variates and not the specific parameters or when there are different distributions or variances involved. The Kruskal-Wallis test (Siegel, 1956) which is based upon the ranking of the variates and the assumption that if different populations are not different from each other, the ranks should be approximately the same (Sokal & Rohlf, 1969).

The number of branches was found to be a useful character by Stuckey (1962). Its interspecific and intraspecific importance was tested with the Kruskal-Wallis test as was petal color. Petal color has also been tested with analysis of variance and the multiple range test. This was done, even though it is not statistically valid, in an attempt to show which populations differed.

Simple regression based on the character means of each population was computed with selected morphologic and ecologic characters. Both interspecific and intraspecific regression were done with all combinations.

Simple regression is a means of measuring a linear relationship between two characters assuming that one is dependent upon the other. The regression establishes the form (positive or negative) and significance of this relationship between two variables. The presence of a significant relationship between two variables should not be assumed to indicate any biologic dependence of one upon another as both variables may be dependent on a third, unknown variable.

Multiple regression (Bliss, 1967) was used on a selected number of morphological characters in an attempt to determine which morphologic and/or ecologic characters seem to be most important in explaining the variation of a particular character. Multiple regression estimates the total relation between one variable and each predicting variable.

Seemingly logical variables (Hart, 1972, Appendix 14) were tested against the one character Y. Those characters seeming most important in explaining the variability of character Y were retested and retested again each time eliminating the least important variable. Upon elimination of those characters which could least explain the variability of Y, one to a few characters will usually remain. Elimination of any of these remaining characters causes a drastic drop in the F value and a decrease in the Coefficient of Determination (r^2) which is the per cent of the variation of Y which the remaining characters explain.

Multiple regression analyses of five ecological characters and each of the morphological characters making up the two major groups of correlated *Cardamine bulbosa* characters (Fig. 38) were attempted. These analyses were performed on the premise that one of these two groups might be more strongly influenced by certain ecologic factors than the other.

A Model I linear regression is used when a dependent or random variable (Y) is correlated with and varies on an independent or fixed variable (X). When both variables are dependent, the rare Model II linear regression is assumed. Computations for both models are identical.

Two similarity coefficients were used in analysis of the twenty populations of *Cardamine douglassii* and *C. bulbosa*. Each population was considered as being an operational taxonomic unit (OTU). The correlation coefficient and distance coefficient were used for comparing the populations based on thirty-one morphological characters. The programs, written in the APL/360 languages according to Sokal and Sneath (1963) and Sneath and Sokal (1973) have the missing data option. Population means of the thirty-one characters were first standardized (Sokal & Sneath, 1963) and then the similarity coefficients were calculated.

The hybrid index was calculated based on four relatively good diagnostic characters. It was felt that these characters (pubescence length, plant height, flower color, and blooming time) were the best characters in distinguishing *Cardamine bulbosa* from *C. douglassii*. In each of these four characters, the typical *C. bulbosa* condition was set at from one to four while *C. douglassii* was set at zero (Fig. 41). Only the plants collected by the random method were used.

It is important to realize that the value given to the fourth character in this study (blooming time) was based on the number of flowers in bloom or yet to bloom divided by the total number of buds, flowers and fruits, when compared to one or the other parental species. Populations from communities in which only one species of *Cardamine* was found were assumed to be typical for that species.

As random samples of the two species were collected at different times without any knowledge of the relative abundance of each of the species, no attempt should be made to predict the relative abundance of the two species from the hybrid index percentages.

In this investigation a number of statistical tests were used to determine and interpret morphological relationships and/or differences.

The APL/360 computer was used with some of the functions written by the authors (TWH) expressly for this study.

To identify the inter- and intrapopulational variation of *Cardamine bulbosa* and *C. douglassii* the analysis of variance and the SNK Multiple Range Test (Table 11) were computed on ten of the thirty-one characters measured. In interpreting (Table 11) each multiple range test, all means which are over any given line are not significantly (0.05 level) different from each other. Means which are not over the same continuous line are significantly different.

Standard deviation, mean and range are plotted for pubescence length, plant height (Figs. 30, 31), the number of hairs per mm^2 (Fig. 32), and the number of cauline leaves (Fig. 33). Pubescence length (Fig. 30) appears to be a character which can always be used to identify an individual plant species. Population number 13 (*Cardamine douglassii*) has a significantly shorter pubescence length (Fig. 30) than 5 of the 7 other populations of *C. douglassii*. The difference in pubescence lengths of plants grown under controlled greenhouse conditions can be seen in Fig. 34. The pubescence length of the known hybrid is noticeably intermediate between that of *C. bulbosa* and *C. douglassii*. Plant height is also a good diagnostic character (Fig. 31). While significant, plant height does show considerable overlap and cannot be used as the only identifying character.

Again, the Bowling Green population of *Cardamine douglassii* (no. 13) is unusual because of its petal index. These plants (Table 11), on the average, have the largest index of all, significantly different from the southern ($n = 32$) populations of that species.

The Student Newman Keuls Multiple Range Test (Table 11) indicates that the length of the second leaf is not an important character distinguishing the two species. How-

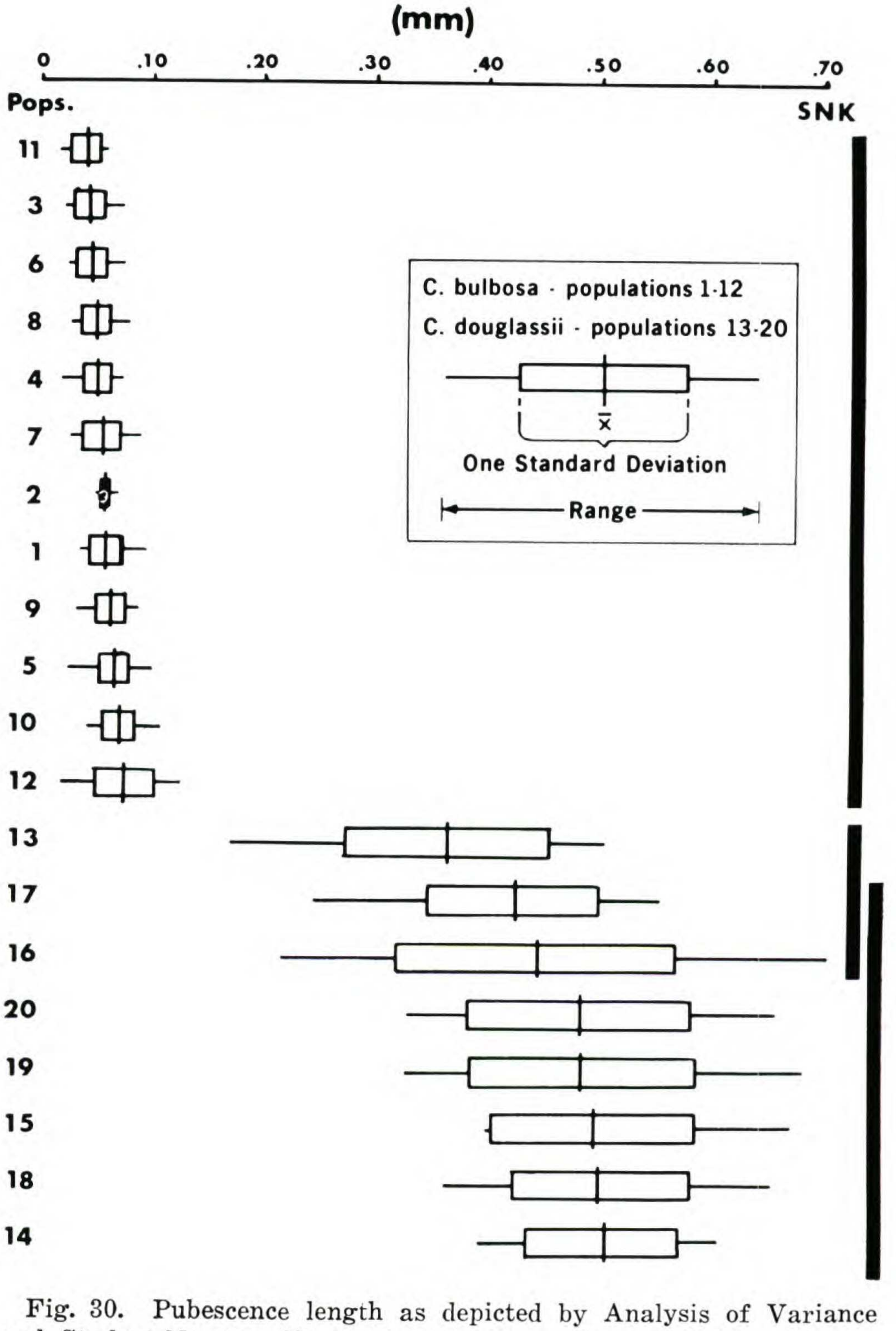


Fig. 30. Pubescence length as depicted by Analysis of Variance and Student-Newman-Keuls Multiple Range Test (SNK).

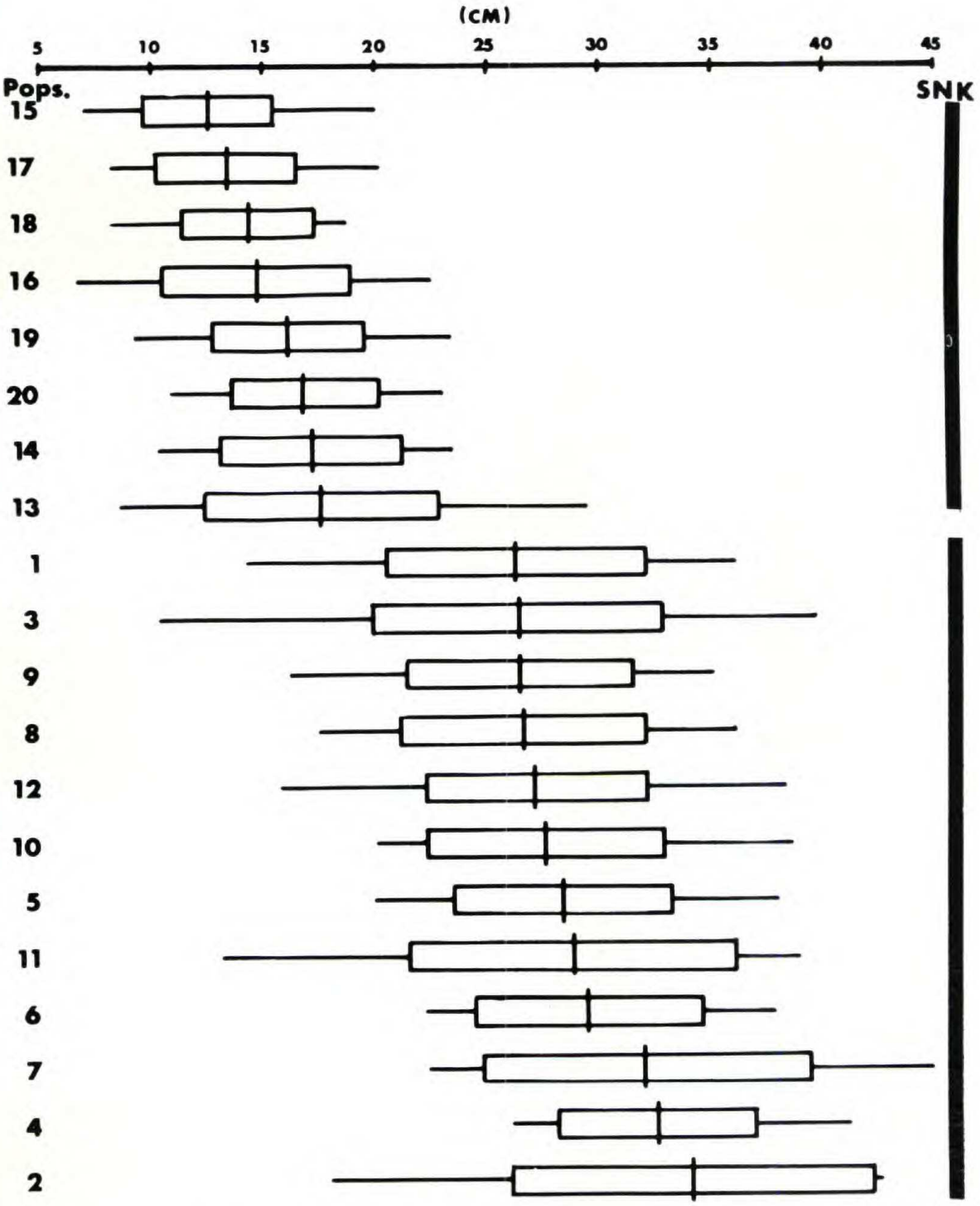


Fig. 31. Plant height as depicted by Analysis of Variance and Student-Newman-Keuls Multiple Range Test (SNK). See Figure 30.

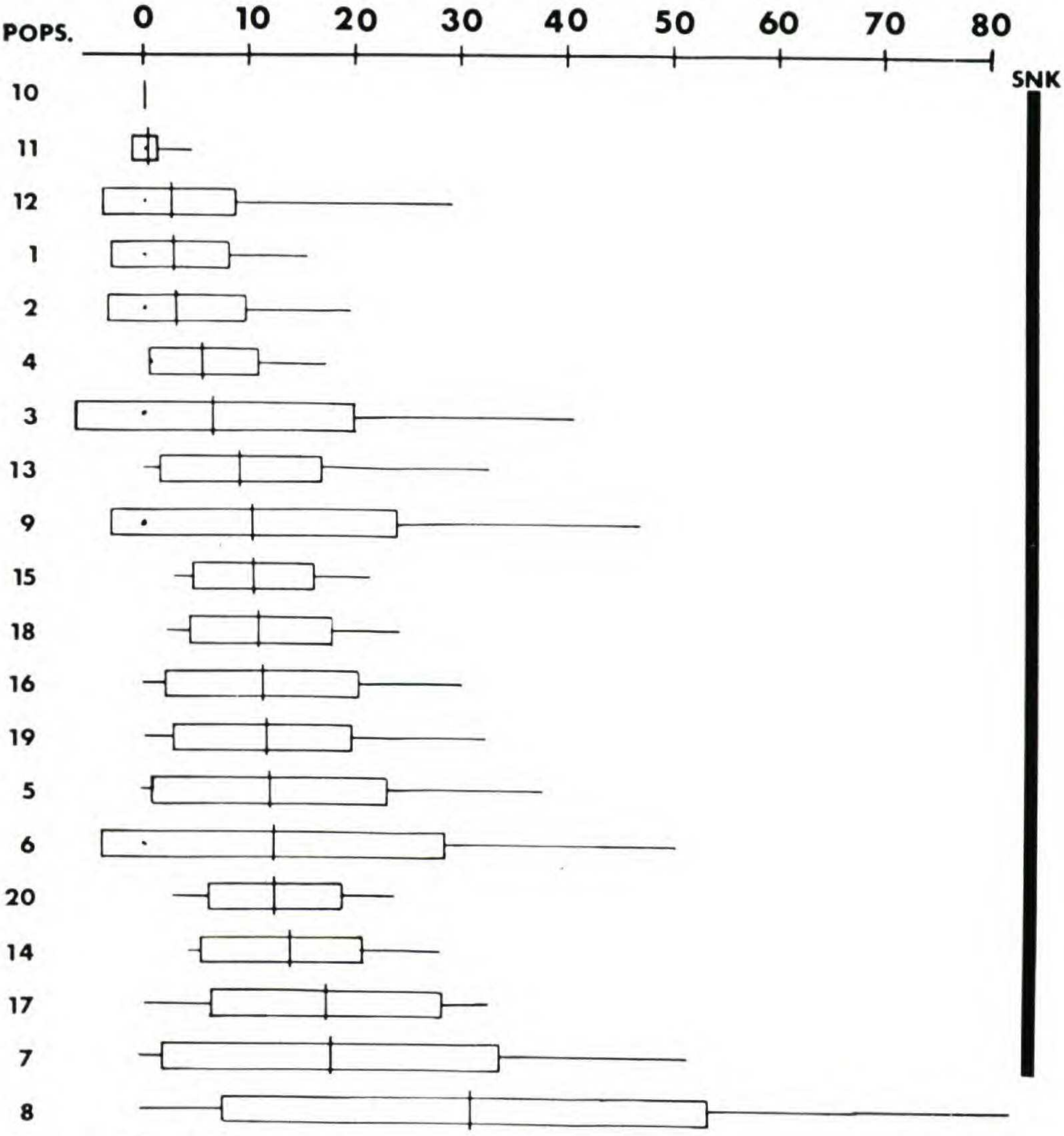


Fig. 32. Number of hairs per square millimeter as depicted by Analysis of Variance and Student-Newman-Keuls Multiple Range Test (SNK). See Figure 30.

ever, all *Cardamine douglassii* populations have shorter average leaves than does *C. bulbosa*. The two northern, polyploid *C. douglassii* populations have larger leaves than the other *C. douglassii*. Forma *fontinalis* of *C. bulbosa* has the longest leaves of all populations studied but unlike the number of leaves (Fig. 33), this character difference proved to be insignificant.

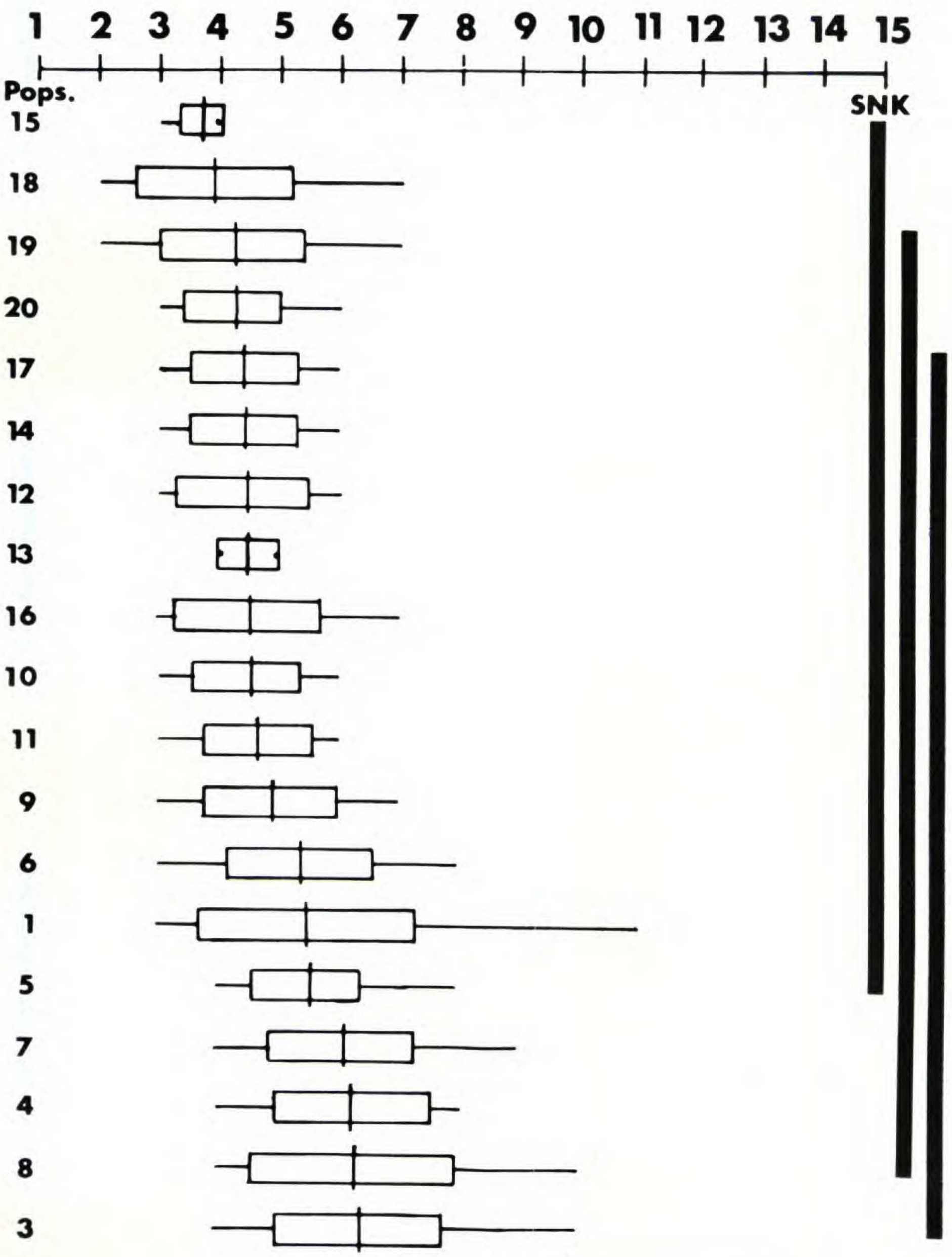


Fig. 33. Number of leaves per stem as depicted by Analysis of Variance and Student-Newman-Keuls Multiple Range Test (SNK). See Figure 30.

TABLE 11. ANALYSIS OF VARIANCE AND STUDENT NEWMAN KEULS TEST (0.05 LEVEL) OF *CARDAMINE BULBOSA* AND *C. DOUGLASSII* USING TEN CHARACTERS.

Character 2	Plant Height											f = 33.2								
Pop. No.	15	17	18	16	19	20	14	13	1	3	9	8	12	10	5	11	6	7	4	2
N	15	20	22	15	23	15	13	30	18	21	22	16	22	19	20	14	17	17	15	8
$\bar{x}$	12.55	13.35	14.3	14.71	16.12	16.75	17.15	17.36	26.27	26.31	26.4	26.5	27.04	27.47	28.25	28.69	29.47	31.9	32.45	34.01

Character 9	2nd Leaf Length											f = 17.37								
Pop. No.	17	18	14	19	20	16	9	15	13	8	10	1	4	3	11	5	7	12	6	2
N	20	22	13	23	15	15	21	15	30	16	19	18	14	21	13	20	17	22	17	8
$\bar{x}$	1.76	1.97	1.99	2.01	2.02	2.05	2.07	2.08	2.29	2.46	2.52	3.12	3.30	3.32	3.44	3.65	3.74	3.77	4.11	4.55

Character 12	Number of Leaves									f = 14.89										
Pop No.	15	18	19	20	17	14	12	13	16	10	11	9	6	1	5	7	4	8	3	2
N	15	22	23	15	20	13	22	30	14	19	14	22	17	18	20	17	15	16	21	8
$\bar{x}$	3.67	3.91	4.26	4.27	4.45	4.46	4.5	4.53	4.57	4.58	4.71	4.96	5.41	5.5	5.6	6.18	6.33	6.38	6.48	10

Character 18	Leaf 2 index									f = 9.422										
Pop. No.	8	9	6	7	10	5	11	12	20	18	14	4	3	19	2	17	16	15	13	1
N	16	21	17	17	19	20	13	22	15	22	13	14	14	23	8	20	15	15	30	18
$\bar{x}$	.36	.41	.41	.42	.45	.46	.46	.47	.48	.50	.50	.50	.50	.51	.55	.55	.58	.63	.65	.83

Character 20	No. of Hairs									f = 7.7										
Pop. No.	10	11	12	1	2	4	3	13	9	15	18	16	19	5	6	20	14	17	7	8
N	19	14	22	18	8	15	21	30	22	15	22	15	23	20	17	15	13	20	17	16
$\bar{x}$	.000	.033	2.52	2.77	3.09	5.62	6.61	9.65	10.2	10.4	10.9	11.4	11.6	12.1	12.5	12.6	13.2	17.6	18.2	30.8

Character 21	Pubescence Length									f = 182.6										
Pop. No.	11	3	6	8	4	7	2	1	9	5	10	12	13	17	16	20	19	15	18	14
N	11	17	17	16	14	17	5	17	18	19	19	22	30	20	15	15	23	15	22	13
$\bar{x}$	4.37	4.49	4.85	5.14	5.28	5.86	6.08	6.12	6.51	6.9	7.19	7.85	36.94	42.58	44.55	48.51	48.64	49.68	50.39	50.81

TABLE 11. (Continued)

Character 24	Petal Length									f = 16.217										
Pop. No.	8	9	6	4	7	5	14	10	19	18	20	16	15	17	11	1	13	3	2	12
N	16	22	17	15	17	20	13	19	23	22	15	15	15	18	12	18	21	21	5	20
$\bar{x}$	7.68	8.04	8.08	8.51	8.61	8.68	9.24	9.30	9.72	9.90	10.06	10.18	10.23	10.34	10.35	10.55	10.70	10.80	11.03	12.32

Character 26	Petal Index									f = 10.37										
Pop. No.	10	20	17	16	11	5	2	19	14	4	18	8	6	9	15	7	12	1	3	13
N	19	15	18	15	12	20	5	23	13	15	22	16	17	22	15	17	20	18	21	1
$\bar{x}$	.378	.397	.403	.403	.416	.416	.416	.417	.425	.427	.428	.435	.467	.474	.479	.494	.50	.530	.537	.591

Character 30	Leaf 2 Width Index									f = 6.336										
Pop. No.	12	14	17	20	15	10	19	18	13	16	5	6	8	9	7	4	11	1	2	3
N	22	13	20	20	15	19	22	22	30	15	19	17	16	22	17	15	14	18	8	30
$\bar{x}$	.764	.782	.796	.801	.803	.805	.813	.821	.827	.837	.847	.863	.871	.872	.906	.926	.938	.943	.955	.981

Character 31	Color									f = 59.6										
Pop. No.	1	2	3	5	6	16	11	4	9	7	12	8	18	19	17	20	14	15	13	16
N	18	8	21	19	17	19	12	15	22	17	20	16	16	22	20	15	13	15	29	15
$\bar{x}$	1	1	1	1	1	1	1	1.13	1.22	1.59	1.65	1.94	3.38	3.77	4.7	4.73	4.85	5.53	5.69	5.87

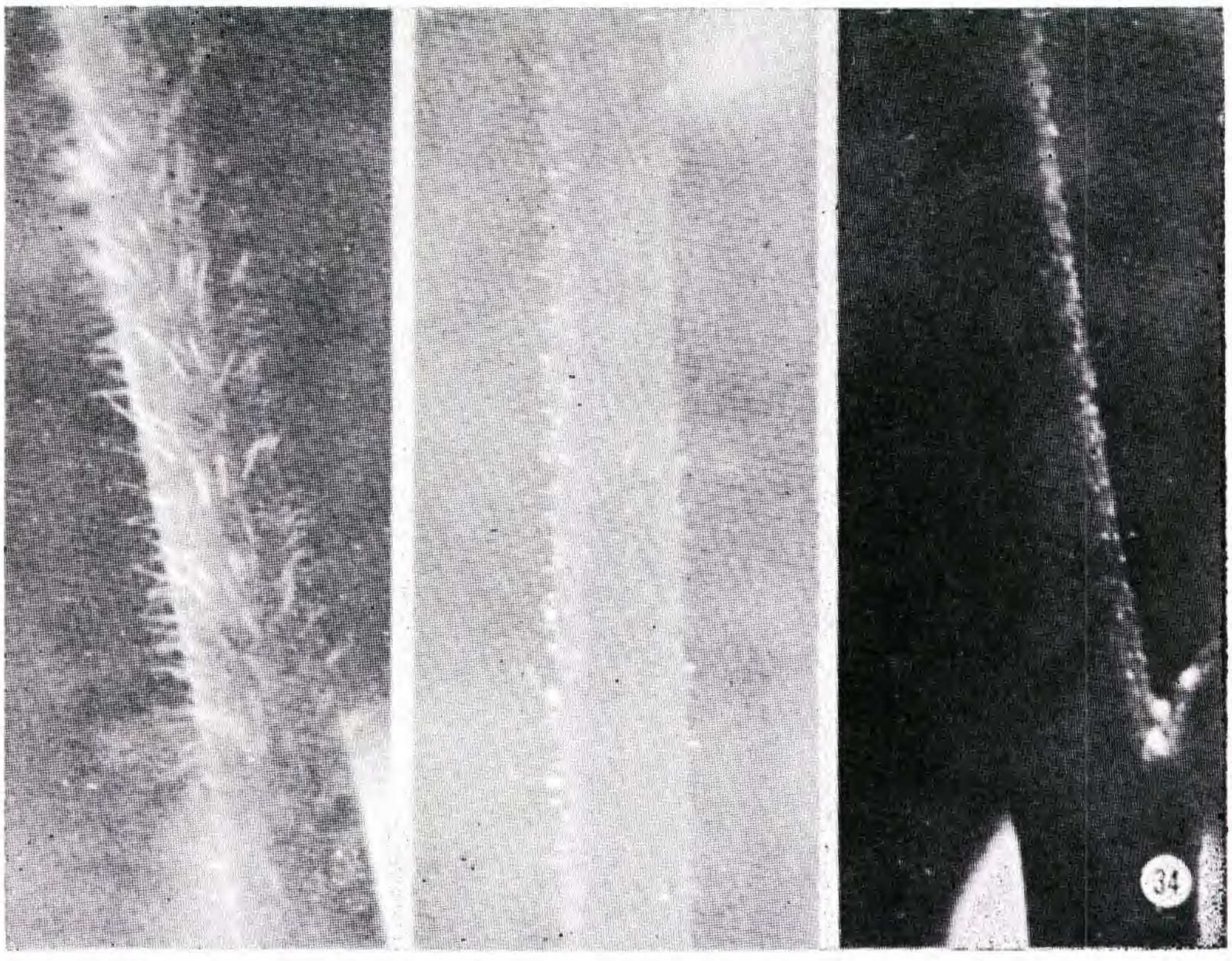


Fig. 34. Left Panel, pubescence in *Cardamine douglassii*. Middle panel, pubescence in known hybrid. Right panel, pubescence in *C. bulbosa*.

Branch number and petal color, two nonparametric characters (no. 1 and 31), were found to vary significantly within the twenty populations studied (Table 11) when tested with the Kruskal-Wallis One Way Analysis of Variance Test. Visualization of the location of the important differences can be made using Figures 35 and 36 where the frequency of the petal color index and the number of branches are shown.

Both *Cardamine douglassii* and *C. bulbosa* generally lack branches. However, four populations of *C. bulbosa* average greater than two branches per plant. The multiple range test, used only to demonstrate populational differences and not as an accurate statistic, shows a species separation by petal color. The flowers of all *C. bulbosa* average white (1) to light pink (2) while *C. douglassii* is darker in color. Three *C. douglassii* populations were significantly darker than all the others.

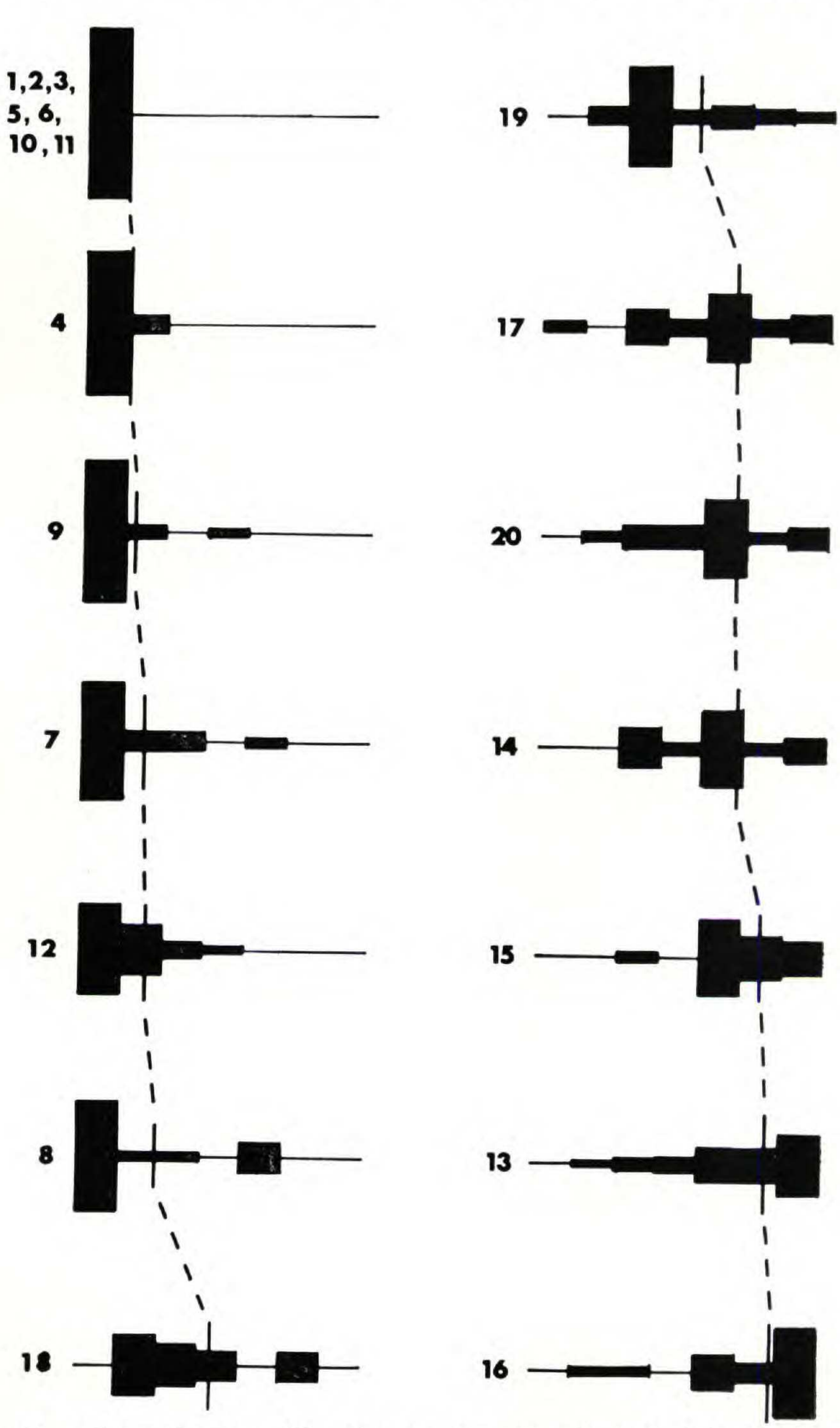


Fig. 35. Color index. The frequency of colors (white = 1, purple = 7) in the population is indicated by the width of the lines. The mean value is indicated by the dotted line. *Cardamine bulbosa* (populations 1-12) and *C. douglassii* (populations 13-20).

TABLE 12. ANALYSIS OF VARIANCE AND MULTIPLE RANGE TEST (0.05) OF *CARDAMINE BULBOSA* USING ELEVEN CHARACTERS.

Character 9				2nd Leaf Length				f = 8.6596				
Pop. No.	9	8	10	1	4	3	11	5	7	12	6	2
N	21	16	19	18	14	21	13	20	17	22	17	8
$\bar{x}$	2.07	2.46	2.52	3.12	3.30	3.32	3.44	3.65	3.74	3.77	4.11	4.55

Character 30				Leaf 2 Width Index				f = 10.36				
Pop. No.	12	10	5	6	8	9	7	4	11	1	2	3
N	22	19	19	17	16	22	17	15	14	11	8	30
$\bar{x}$	.764	.805	.847	.863	.871	.872	.906	.925	.938	.943	.955	.981

Character 24				Petal Length				f = 20.53				
Pop. No.	8	9	6	4	7	5	10	2	11	1	3	12
N	16	22	17	15	17	20	19	6	12	18	21	20
$\bar{x}$	7.68	8.04	8.08	8.51	8.61	8.68	9.30	9.41	10.35	10.55	10.8	12.3

Character 21				Pubescence Length				f = 7.76				
Pop. No.	11	3	6	8	4	7	2	1	9	5	10	12
N	11	17	17	16	14	17	5	17	18	19	19	22
$\bar{x}$	4.36	4.49	4.85	5.14	5.28	5.86	6.07	6.12	6.5	6.9	7.19	7.85

TABLE 12. — Continued.

Character 12				Number of Leaves				f = 12.7				
Pop. No.	12	10	11	9	6	1	5	7	4	8	3	2
N	22	19	14	22	17	18	20	17	15	16	21	8
$\bar{x}$	4.5	4.58	4.71	4.95	5.41	5.5	5.6	6.18	6.33	6.37	6.48	10

Character 20				No. of Hairs				f = 9.08				
Pop. No.	10	11	12	1	2	4	3	9	5	6	7	8
N	19	14	22	18	8	15	21	22	20	17	17	16
$\bar{x}$	.000	.033	2.25	2.77	3.09	5.62	6.61	10.2	12.1	12.5	18.2	30.8

Character 18				Leaf 2 Index				f = 14.91				
Pop. No.	8	9	6	7	10	5	11	12	4	3	2	1
N	16	12	17	17	19	20	13	22	14	21	8	18
$\bar{x}$	.36	.410	.411	.416	.449	.456	.461	.472	.502	.503	.546	.831

Characters 2, 26, 29 and 31 not significant

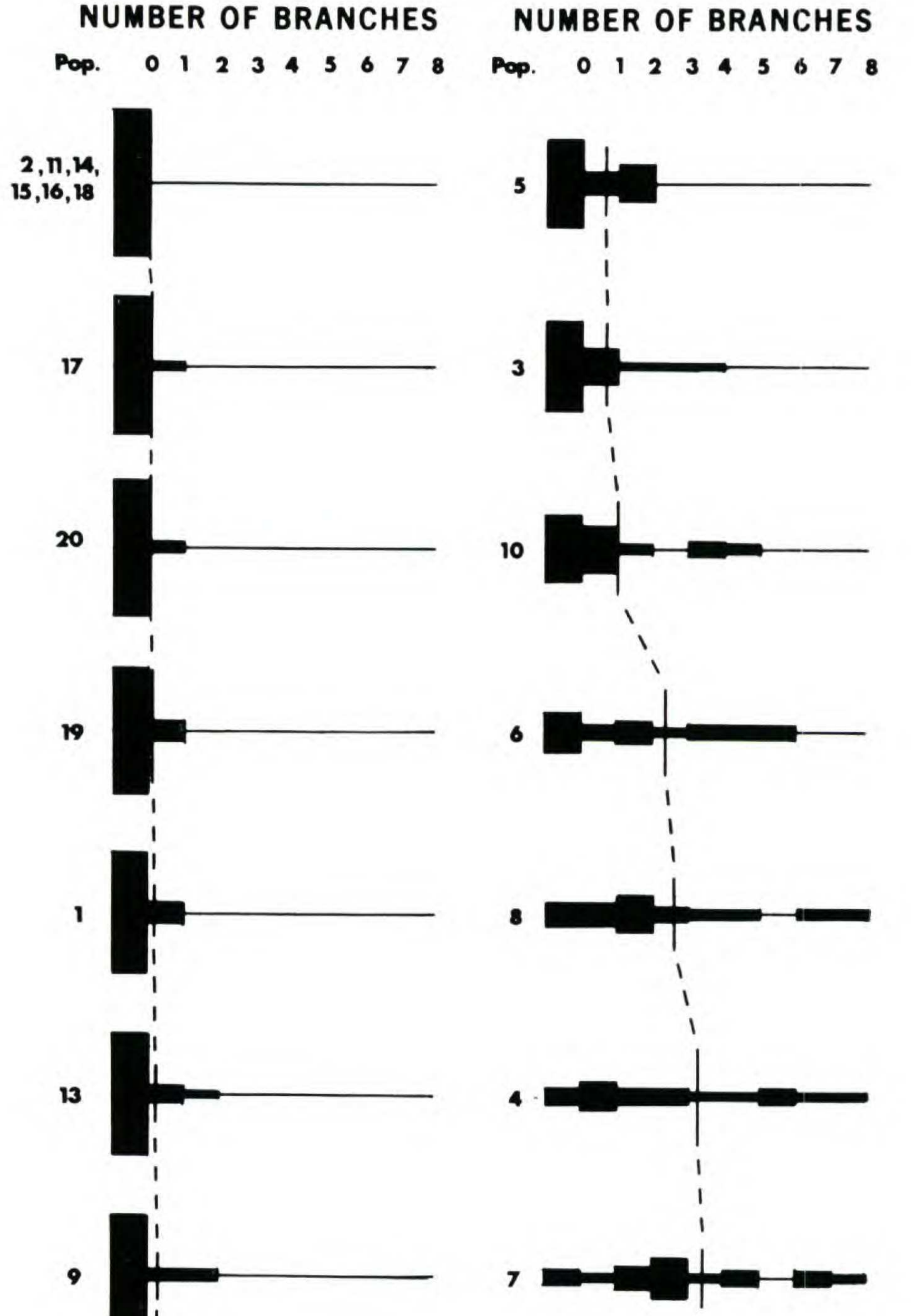


Fig. 36. Number of branches. The frequency of the number of branches in the population is indicated by the width of the line. See Figure 35.

Population differences within *Cardamine bulbosa* were measured using analysis of variance (See Hart, 1972) and a multiple range test (Table 12). Population number 12 has at least two significant differences from all the populations studied (Fig. 37). This population is near Bowling Green, Ohio and consists of scattered clumps of *C. bulbosa* among large numbers of *C. douglassii.* Putative hybrids are relatively common and can be identified by their intermediate flowering time and length of pubescence. They are, however, not included in the statistical study of the populations. Nevertheless, population 12 (Fig. 37) is different from all other populations of *C. bulbosa,* and it varies towards *C. douglassii* in pubescence length, flower color and number of leaves. Population 12 (*C. bulbosa*) differs from most other populations of *C. bulbosa* studied as to petal length and leaf width index of the second leaf.

A second population (no. 8) is outstanding because it differs from other *Cardamine bulbosa* populations (Fig. 37) in having numerous branches, pink petals (Fig. 35) and is a member of chemical race C. It is possible that growth in full sunlight may have accentuated other differences such as the amount of pubescence, petal size, filament length, and pubescence length.

Finally, population 2 is notably different (Fig. 37) from five or more of the other *Cardamine bulbosa* populations. This population of *C. bulbosa* grows almost entirely submerged in cold spring water and was collected in Missouri.

Simple regressions of seventeen morphological characters, using population averages as well as six ecologic measurements, were computed in all possible combinations. Intrapopulational correlations studied by this method indicate that within *Cardamine douglassii* five characters are significantly (0.05) correlated with chromosome number. These characters are the leaf index, number of hairs per sq. mm, average pubescence length, stem width, and the petal index. There is also an indicated relationship of the filament length, stem width and petal color with various ecologic factors. No important or unexpected character

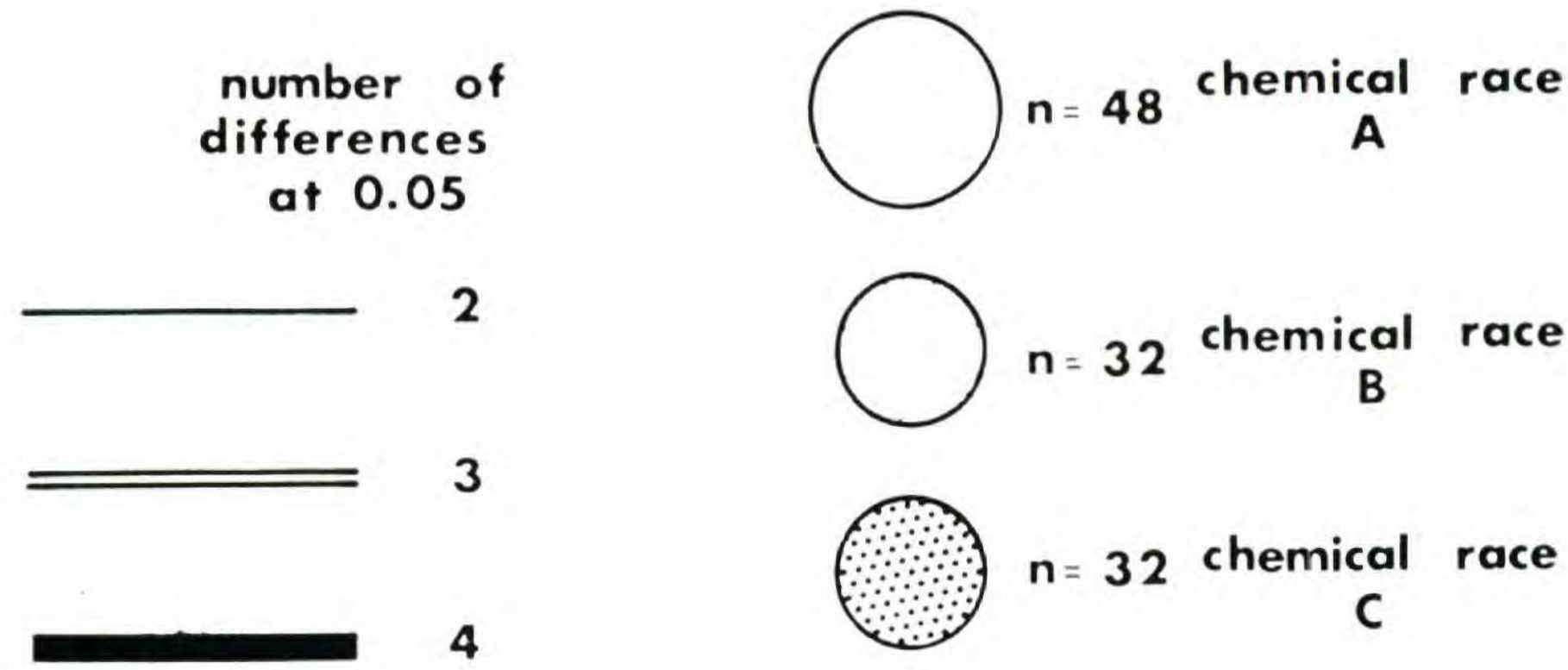
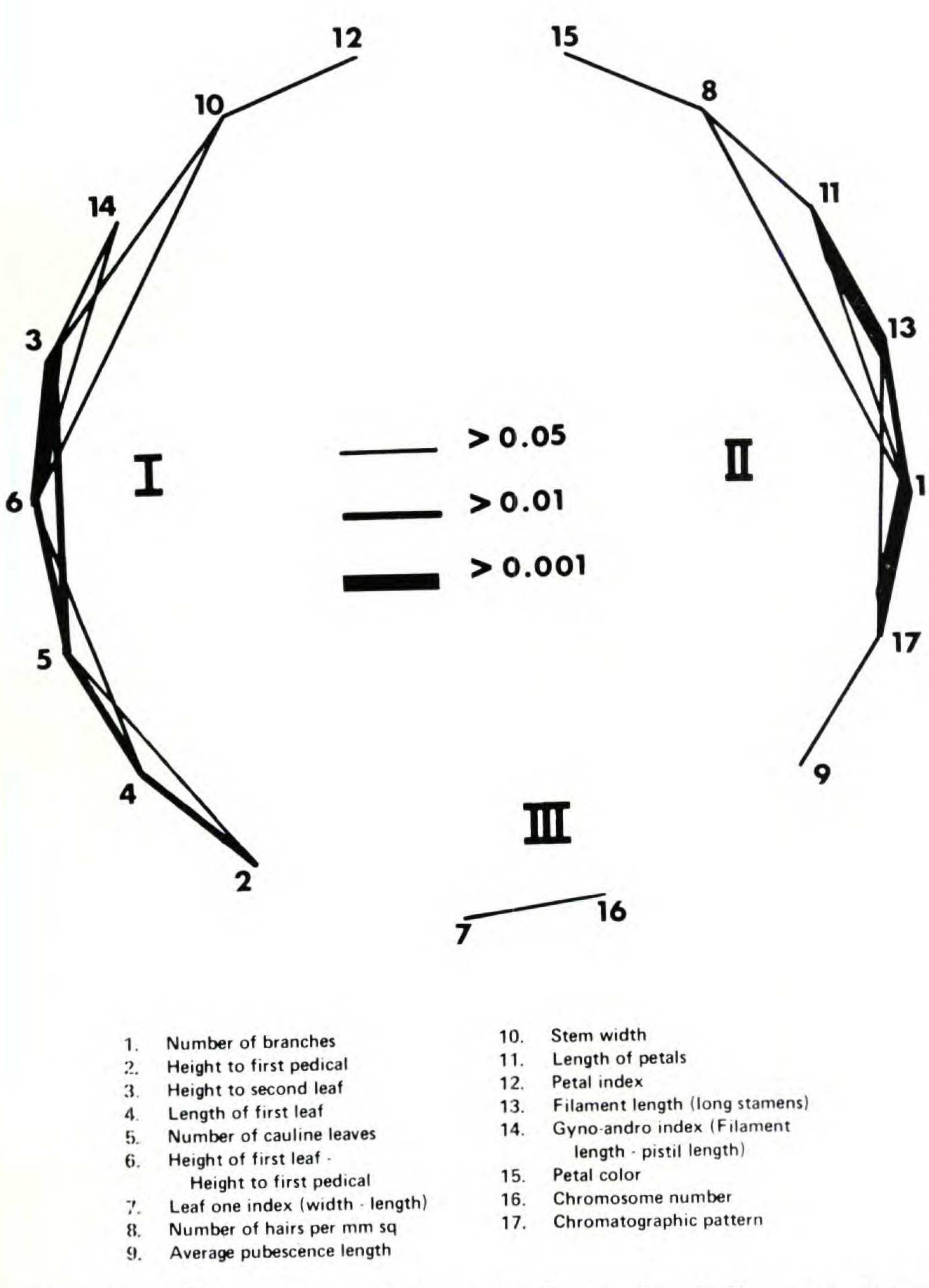


Fig. 37. Summary of the differences (eleven characters) between populations of *Cardamine bulbosa* as shown by the Multiple Range Test.



1. Number of branches
2. Height to first pedical
3. Height to second leaf
4. Length of first leaf
5. Number of cauline leaves
6. Height of first leaf - Height to first pedical
7. Leaf one index (width - length)
8. Number of hairs per mm sq
9. Average pubescence length
10. Stem width
11. Length of petals
12. Petal index
13. Filament length (long stamens)
14. Gyno-andro index (Filament length - pistil length)
15. Petal color
16. Chromosome number
17. Chromatographic pattern

Fig. 38. Character correlations in *Cardamine bulbosa* as shown by simple regression analysis.

correlations were seen when all populations were included in the interpopulational simple regression analysis.

Certain of the twenty-five significant character correlations of *Cardamine bulbosa* as measured by simple regression are of particular interest. The chromatographic pattern, for example, is correlated with branch number, pubescence length, and filament length.

Figure 38 shows all the significant correlation (0.05) levels of the various morphological characters. These correlations appear to be in three groups. The first group is the chromatographic pattern and its associated characters. A second group is those characters dealing with size and the various indices. The third relatively small group indicates a correlation between chromosome number and the leaf index. The multiple regression of the correlated characters, within *Cardamine bulbosa*, with various ecologic factors showed that no significant correlations occurred.

The shaded correlation matrix (Fig. 39) based on twenty populations shows *Cardamine douglassii* (populations 13-20) as being quite similar while *C. bulbosa* (populations 1-12) shows considerable variation within the species. Populations 13 and 14 show a little less similarity than is typical for the other *C. douglassii* groups (Fig. 40). Both of these populations are growing in moist soil with *C. bulbosa* and putative hybrids nearby. However, populations 13 and 14 have different chromosome numbers and are found in different geographical sites in Ohio.

OTU number 12 is a population of *Cardamine bulbosa* growing in northern Ohio and apparently hybridizing with *C. douglassii*. Population number 12 is not very similar to either species.

The distance coefficient failed to produce as sharp a separation between *Cardamine douglassii* (OTU 13-20) and *C. bulbosa* (OTU 1-12) as might be anticipated. OTU's number one and two are not very similar to most other *C. bulbosa*. In both these populations the plants were growing partially submerged in cold spring water. OTU one and ten also have a chromosome number of $n = 48$.

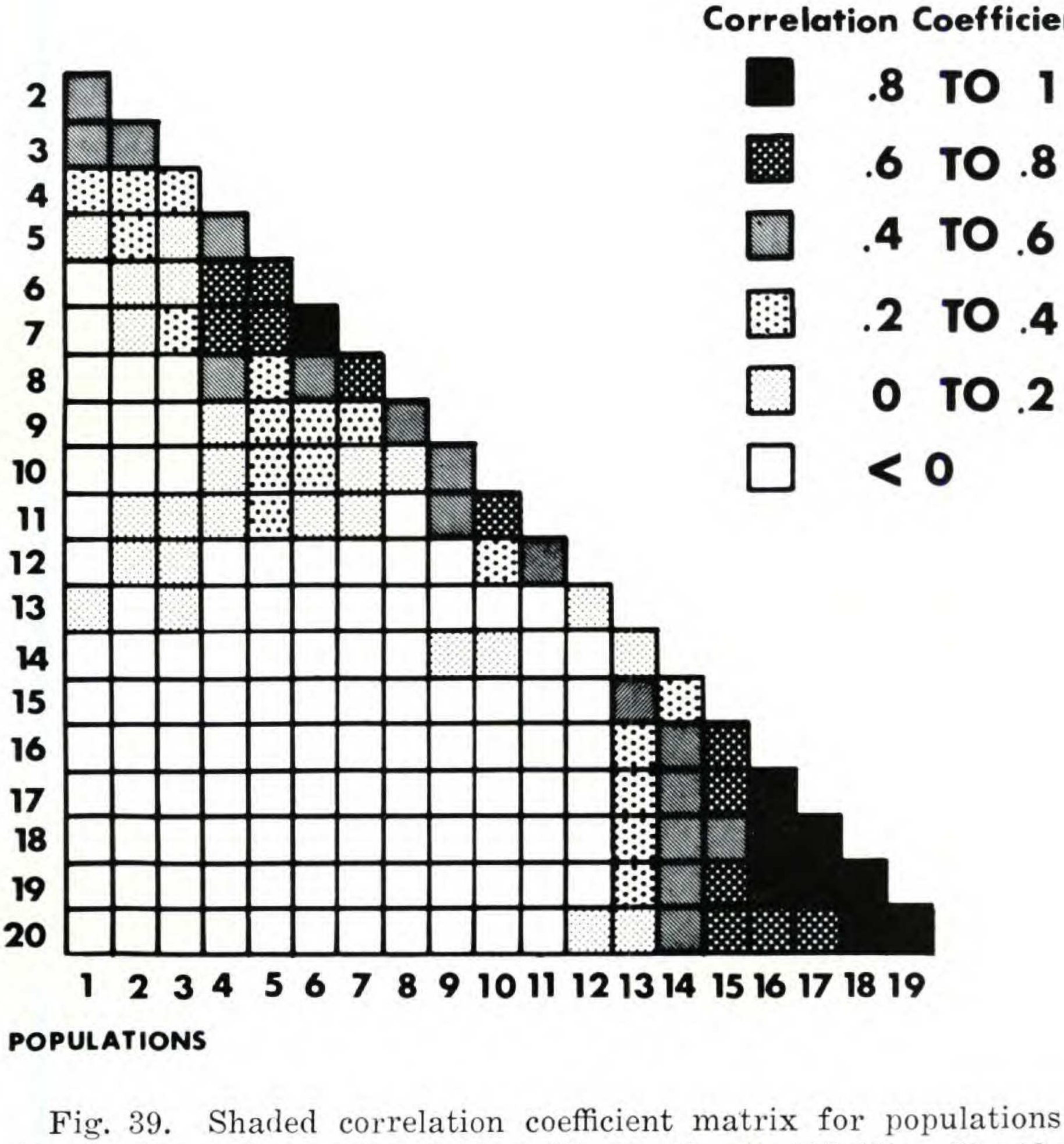


Fig. 39. Shaded correlation coefficient matrix for populations of *Cardamine bulbosa* (1-12) and *C. douglassii* (13-20). Significant correlation (0.05) equals 0.355.

Plants from community number two have previously been classified as *C. bulbosa* forma *fontinalis* (Palmer & Steyermark, 1938).

The hybrid index (Table 13, Fig. 41) indicates that with the exception of populations 12 and 13 (the Bowling Green, Ohio populations) the two species are completely separate (Figs. 41, 42). The morphologic and phenologic characters separating the species, however, break down in populations 12 and 13 (Figs. 41, 43).

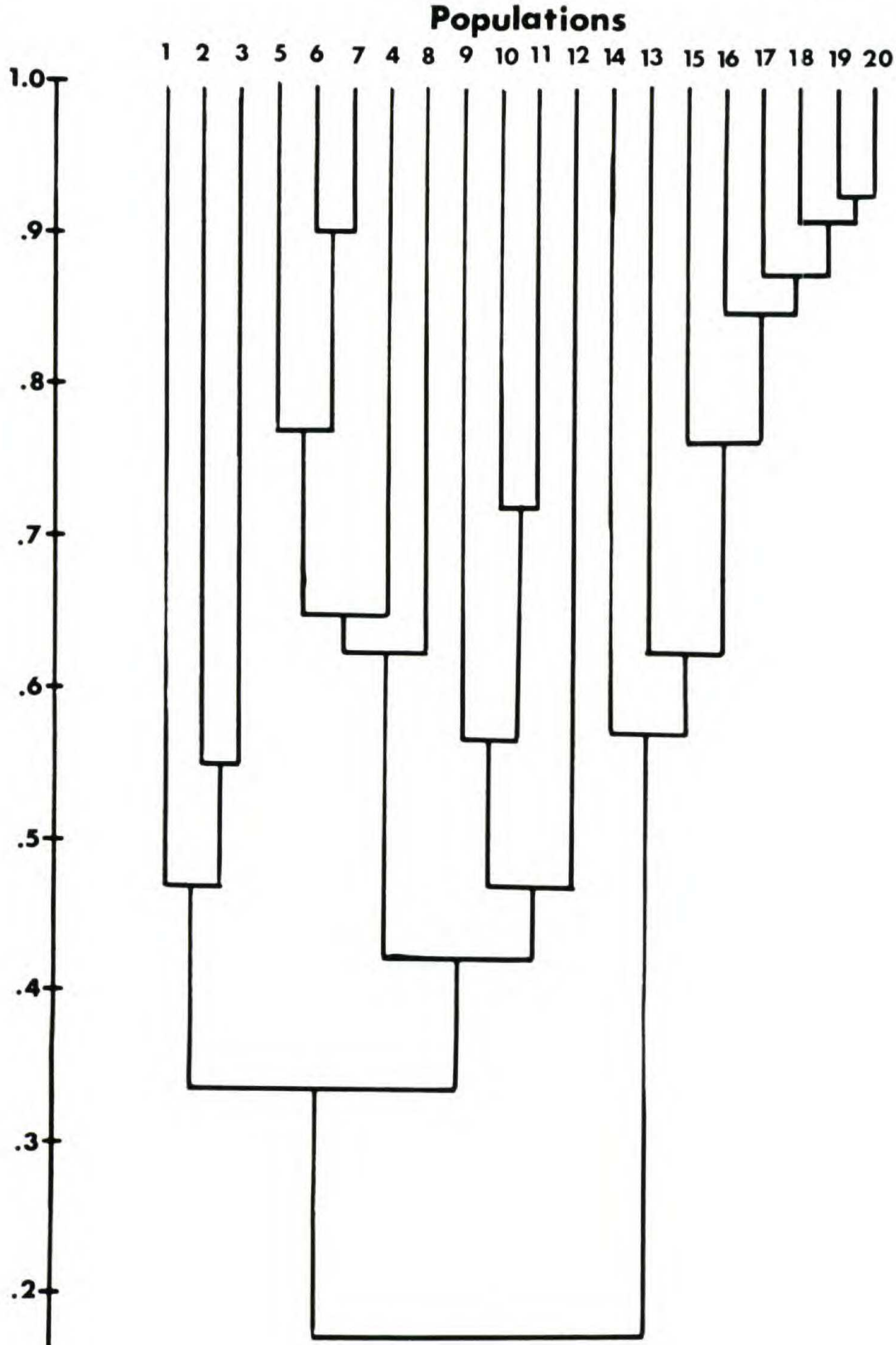


Fig. 40. Correlation phenogram of populations of *Cardamine bulbosa* (1-12) and *C. douglassii* (13-20) with clustering based upon the highest mutual correlation. Significant correlation (0.05) equals 0.355.

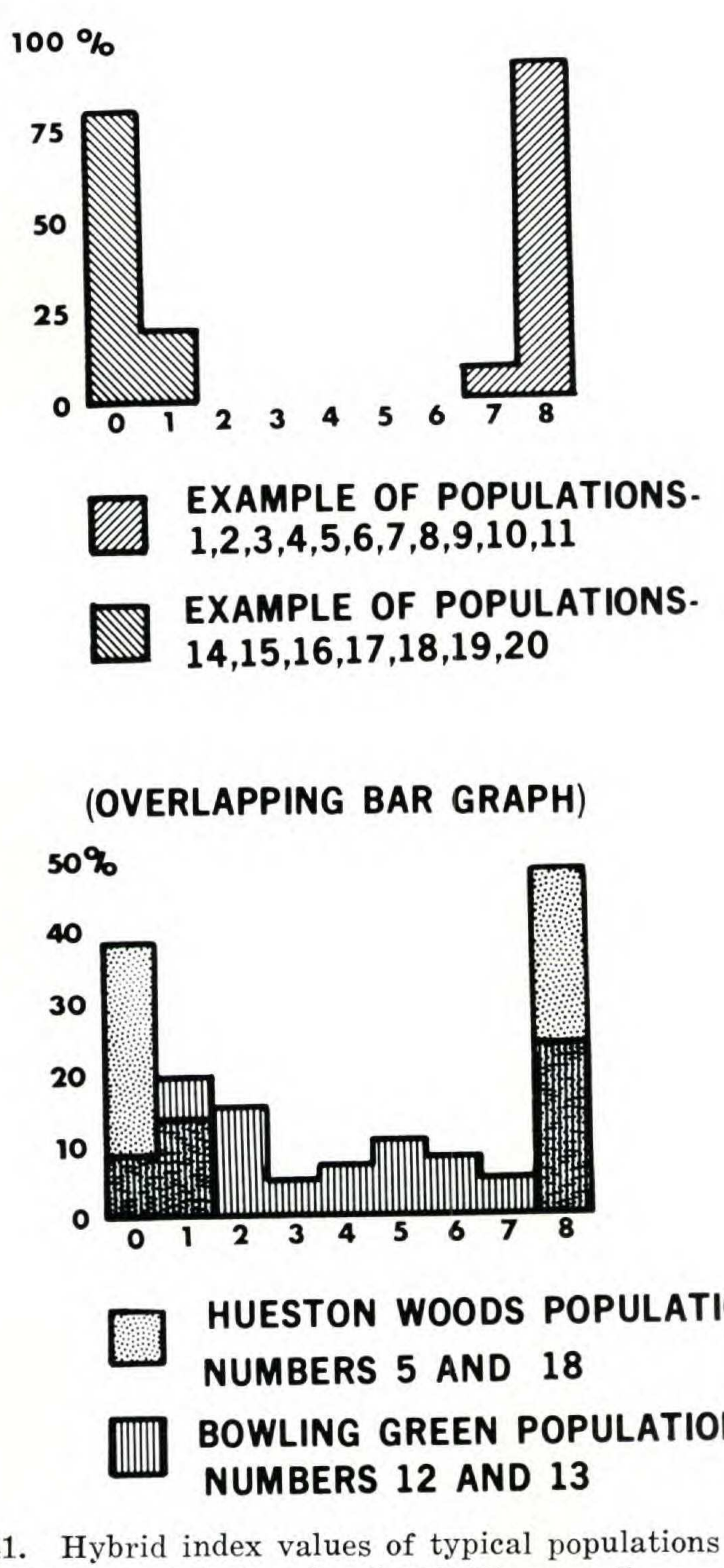


Fig. 41. Hybrid index values of typical populations of *Cardamine bulbosa* (1-12) and *C. douglassii* (13-20) as well as a hybrid swarm from Bowling Green, Ohio.

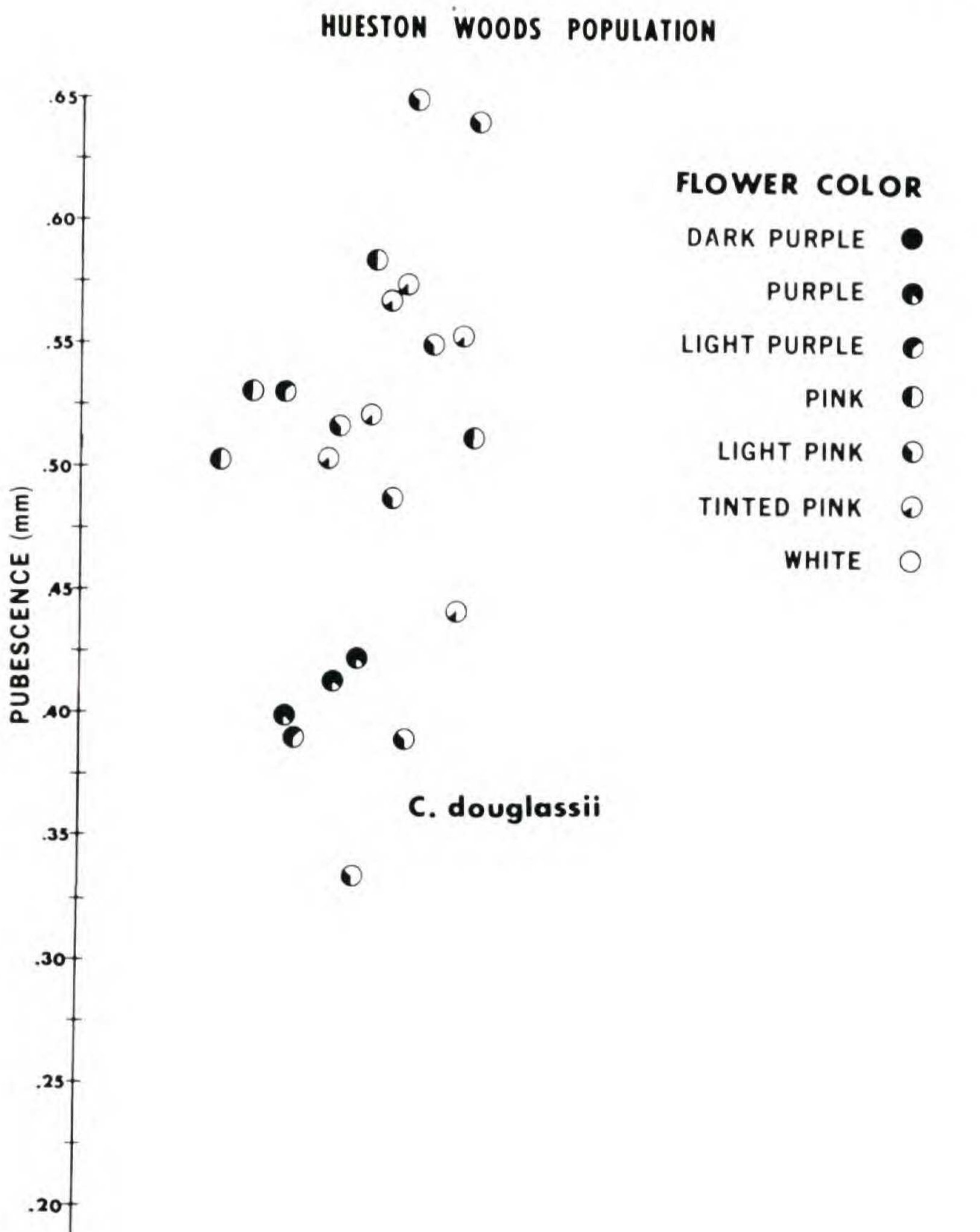


Fig. 42. Scatter diagram depicting the relationship of *Cardamine bulbosa* and *C. douglassii* from the Hueston Woods populations (Nos. 5 and 18).

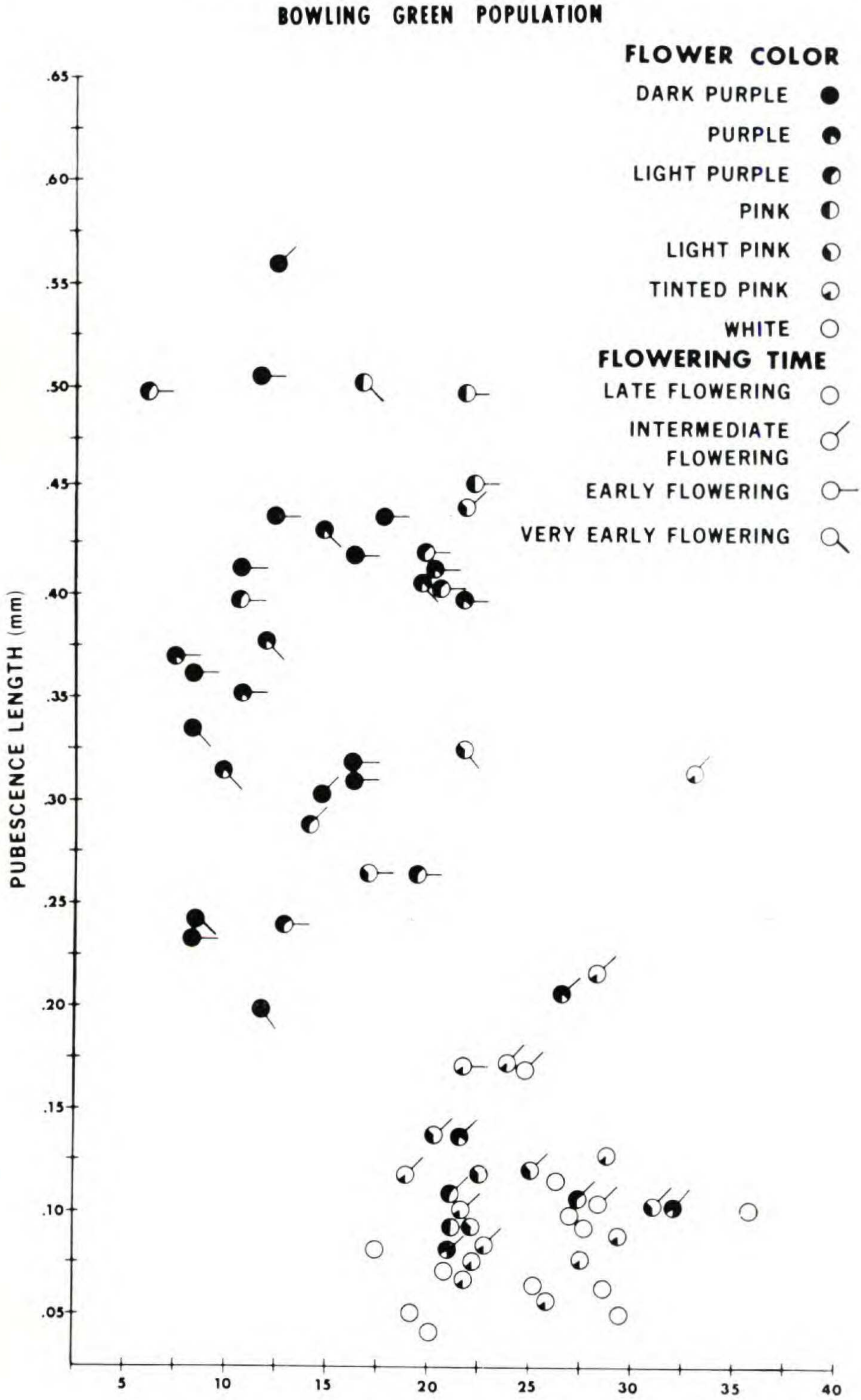


Fig. 43. Scatter diagram depicting the relationship of *Cardamine bulbosa* and *C. douglassii* from the Bowling Green populations (Nos. 12 and 13).

TABLE 13. HYBRID INDEX PERCENTAGES OF POPULATIONS OF *CARDAMINE BULBOSA* AND *C. DOUGLASSII*

Population	Hybrid Index Percentages								
	0	1	2	3	4	5	6	7	8
1	—	—	—	—	—	—	—	17	83
2	—	—	—	—	—	—	—	13	87
3	—	—	—	—	—	—	—	15	85
4	—	—	—	—	—	—	—	—	100
5	—	—	—	—	—	—	—	—	100
6	—	—	—	—	—	—	—	—	100
7	—	—	—	—	—	—	—	18	82
8	—	—	—	—	—	—	—	38	62
9	—	—	—	—	—	—	—	10	90
10	—	—	—	—	—	—	—	—	100
11	—	—	—	—	—	—	—	8	92
12, 13	8	19	15	5	7	11	8	5	23
14	53	47	—	—	—	—	—	—	—
15	93	7	—	—	—	—	—	—	—
16	60	40	—	—	—	—	—	—	—
17	80	20	—	—	—	—	—	—	—
18	72	28	—	—	—	—	—	—	—
19	78	22	—	—	—	—	—	—	—
20	67	33	—	—	—	—	—	—	—

Morphologically the best diagnostic characters for separating *Cardamine bulbosa* from *C. douglassii* (Figs. 44, 45, 46) are pubescence length (Fig. 30), plant height (Fig. 31) and flower color (Fig. 35).

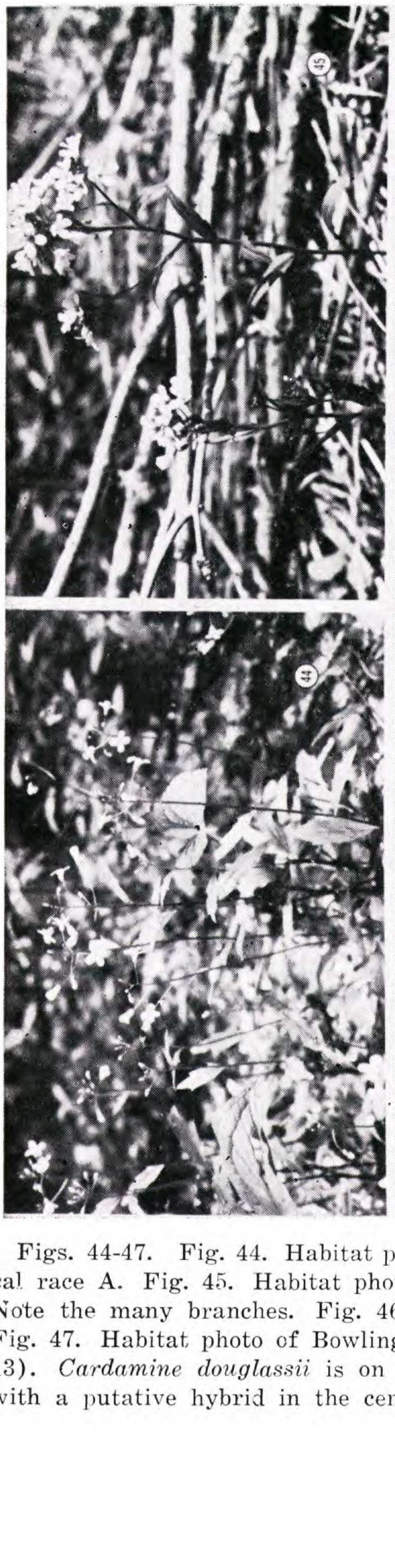
There is no overlap in pubescence length between the two species under normal conditions. *Cardamine bulbosa* normally has hairs less than .15 mm long while normal *C. douglassii* has hair lengths greater than .2 mm (Fig. 34). Problems do arise in that plants with hair lengths falling between .12 and .25 mm do occur (Figs. 34b, 43). These plants often are intermediate in other characters such as flowering time, flower color, and plant height. Throughout this study, those plants with intermediate hair length and flowering time have been considered to be putative hybrids.

Putative hybrids between the two species appear to be relatively rare; however, a few have been found at the John Bachelor Preserve (pop. 19) near Oxford, Ohio, and one at Camp Hook (pop. 14) near Franklin, Ohio. Large numbers of putative hybrids (Fig. 47) were found near Bowling Green, Ohio (populations 12 and 13).

Cardamine douglassii plants at the Bowling Green location (population 13) are somewhat different from most of the other populations of *C. douglassii* studied. They are somewhat like *C. bulbosa* in that the pubescence is shorter and the plants are taller than most *C. douglassii* populations. Bowling Green *C. douglassii* also have a greater petal index and length than do most of this species.

In this same location *Cardamine bulbosa* is found interspersed among the *C. douglassii* plants (Fig. 47). The plants of *C. bulbosa* at this locality have very large petals, many of which tend to be pinkish in color. This may be an indication of introgression or heterosis due to hybridization. In addition these plants have longer pubescence and fewer cauline leaves than most populations of *C. bulbosa*. These species are growing intermixed in this location and each is revealed to be different from other populations of the same species. These aforementioned factors and the presence of large numbers of putative hybrids (Figs. 41, 43) at this locality are strong indications of relatively large amounts of past or present gene flow between the two species in this community.

Dr. John Beaman, Michigan State University (personal communication) reported that *Cardamine bulbosa* and *C. douglassii* differ in their leaf index. This possibility was investigated and could not be substantiated for the plants studied (Table 11, char. 18). However, a correlation between leaf index of the first leaf and pubescence length and petal color was discovered. A comparison of populations 12 and 13 with other *C. bulbosa* and *C. douglassii* populations suggests the presence of introgressive hybridization in the Bowling Green, Ohio population (Heiser, 1973).



Figs. 44-47. Fig. 44. Habitat photo of *Cardamine bulbosa*, Chemical race A. Fig. 45. Habitat photo of *C. bulbosa*, Chemical race C. Note the many branches. Fig. 46. Habitat photo of *C. douglassii*. Fig. 47. Habitat photo of Bowling Green, Ohio populations (12 and 13). *Cardamine douglassii* is on the left, *C. bulbosa* on the right, with a putative hybrid in the center.

A previous report of the petal size of *Cardamine douglassii* exceeding that of *C. bulbosa* (Stuckey, 1962) does not appear valid. The three largest petal averages were found in *C. bulbosa* (Table 11). The largest petals were found in populations of *C. bulbosa* high in water content with the exception of the Bowling Green population (no. 12). While not statistically significant, it is interesting to note that four of the five populations having the smallest petals are found within *C. bulbosa* having numerous branches.

Stuckey (1962) mentioned the possibility that *Cardamine bulbosa* (Muhl.) B.S.P. var. *hirsuta* O. E. Schulz was similar to some of the pubescent forms from southwestern Ohio. He may have been referring to certain plants of a race characterized by: numerous branches, the chemical race C pattern, numerous stem hairs, short hairs and smaller flowers with a tendency to have pinkish petals. Numerous branches occur in this race of *C. bulbosa* but not in the species as a whole (Figs. 44, 45). Therefore, this character should not be used as a diagnostic species characteristic, as has been done previously.

Leaf index is correlated with chromosome number in both *Cardamine bulbosa* and *C. douglassii*. However, one cannot tell the chromosome number of a plant by examination of the leaf index due to the overlap involved.

POPULATION FITNESS

An intriguing method for the measurement of the degree of fitness and the homogeneity of populations in their respective environment was presented by Davidson and Dunn (1967). Their method expects a high variability (many uncorrelated factors) in an undisturbed or ancestral situation. They assume that in an environment favorable to the growth of the species there will be more plant to plant variability than in a marginal environment.

The Davidson and Dunn (1967) model imagines species X as being comprised of a number of biotypes (different

genetic combinations) which occupy an environment at a given point in time. The biotypes that are most fit in that environment are likely to contribute the greatest number of genes to the subsequent generation. As selection will tend to reduce the potential variability contained in the biotypes, those biotypes most fit or best adapted to the environment can be thought of as having the potential for higher variability and as having reached an adaptive peak in that particular environment.

If elements of Species X invade a peripheral or ecologically new environment, they may produce a founder population (Mayr, 1942) effectively isolated from other members of the species. Davidson and Dunn (1967) have proposed that: 1) The number of genes incorporated into the new population will be directly proportional to the number which originally entered the population and 2) inversely proportional to the amount of selection pressure for fitness. The number of genes in the founder population is likely to be less than in the original populations, because fewer genes are likely to be introduced, and the selection pressures will probably be different in the new environment. The exact genes contributing to the founder population will depend on which genes entered the populations, the amount of selection, and upon the particular gene(s) affected by selective pressures peculiar to the new environment. Only a portion of the parental genes are introduced and the new population is exposed to different environmental pressures. It is unlikely, therefore, that the new adaptive peak of the founder population will be the same as the parent population.

They conclude that the genetic make-up of the founder population will be different and more homogeneous or less variable than in the original population. This is in agreement with Mayr (1963) who refers to the founder principle saying that "total genetic variation tends to decrease as local populations encounter presumably new selective forces in environments and habitats other than those occupied earlier by predecessor populations."

The founding population, in time, may become more variable through genetic changes. The rate of recovery will depend on the number of sets and kinds of genes which are found in the population as well as time, chance, and the amount of selection against the mutants and recombinants. This polygene recovery may be rapid at times.

The genetic variation may also be considered to vary depending on the environmental features influencing the various characters. Studies treating environmental features as independent variables and morphological characters as dependent variables are relatively common (Hopkins, 1938; Epling & Dobzhansky, 1942; Brown, 1957; Birch *et al.*, 1963). These studies indicate many linear relations between specific environmental and various populational features.

It is usually assumed that genetically different migrants invade selectively different habitats, and changes under these differing conditions are unique in each. Selection by one environment may directly affect genes while under a different environmental selection different genes may be affected.

As a means of measuring the differences between the genetic variability of different populations caused both by evolutionary history (founders principle) and environmental selection, Davidson and Dunn (1967) recommend the use of the correlation coefficient. Upon making random samples, assuming equal variances for the characters, the correlation coefficient is a measure of how close the characters come to having a linear relationship with each other.

It is assumed that the greater the number of correlated characters or the less plant to plant variability they exhibit, the younger the population and/or the higher the selection pressure that plants in this population are under.

Nineteen populations were included in this study with fifteen to twenty plants per population being studied. The population from Pulltight Springs, Missouri, was not included due to the lack of sufficient specimens. Twenty characters were used (Hart, 1972, Appendix 15). These

particular characters were studied since many are important species characters and they appear to be normally distributed.

Difficulties were encountered with plants from the Bowling Green population since some could not be positively identified to species due to the hybridization occurring in this area. Only specimens considered to be pure were used in this test. Had the putative hybrids and backcrosses progeny been included, the variability of both species might have been increased.

A program using the APL/360 computer first standardized all characters and then computed the correlation coefficients (Hart, 1972, Appendix 3). The degrees freedom used were (n — 2) with n equalling the lowest number of measurements used in the computation of the correlation coefficients. Thirteen degrees of freedom were used as the smallest number of plants studied in the populations included here was fifteen.

A measure of within-population correlation magnitude was calculated using the i coefficient of Davidson and Dunn (1967).

The Davidson and Dunn coefficients (Table 14) for pure populations of *Cardamine douglassii* and *C. bulbosa* are similar with both the highest and lowest coefficients found in populations of *C. bulbosa*. As the i values of the populations of *C. douglassii* tend to be slightly higher than those of *C. bulbosa*, one might suspect *C. douglassii* to be the derived species. The different chromatographic patterns of *C. bulbosa* have variable i values, thus failing to indicate which of the races is younger.

The Davidson and Dunn method indicates that *Cardamine douglassii* is the derived species, although no definite conclusions can be reached in this example with reference to the founders principle. The intraspecific variation noticed may be due mostly to environmental selection. *Cardamine douglassii* has three populations with relatively high i values. The Bowling Green population where hybrids are common is intermediate in its i value. If a breakdown of

TABLE 14. INTRAPOPULATION VARIABILITY USING THE DAVIDSON AND DUNN i VALUE

Population Number	Chromosome Number	Chemical Race	Average percent water	i value DF = 13
Cardamine douglassii				
14	32	A	87	26
16	32	A	51	26
15	48, 72	A	84	32
13	48	A	100	37
18	32	A	60	37
19	32	A	33	39
20	32	A	57	40
17	—	A	71.8	53
Cardamine bulbosa				
4	32	C	100	14
5	32	A	60	17
12	32	A	100	22
6	32	C	100	24
9	32	A	100	24
7	32	C	67	25
3	—	A	100	31
1	48	A	100	37
8	32	C	100	43
11	—	—	100	48
10	48	—	46	59

the species themselves is occurring in this location, one might expect much higher variability than is indicated by the i value.

Water has commonly been thought to be an important factor separating these two species (Stuckey, 1962; Gleason & Cronquist, 1963) and it should be noted that in the Bowling Green (no. 13) population the soil is extremely wet, possibly too wet for high variability of *Cardamine douglassii*. The population at the John Bachelor Preserve (no. 19) exhibits the reverse situation with the presence of a sandy soil which may be too dry for this species. The Rush Run population (no. 17) is located in a highly disturbed, moist upland woods. All populations with a high i value differ from most other populations of *C. doug-*

lassii by being either unusually wet, dry or in a disturbed community.

One population of *Cardamine bulbosa* located near Ugly Creek in Mammoth Cave National Park is much less variable than the others. This population appears to be under high selection pressure causing the variables to be highly correlated. The soil is quite dry for *C. bulbosa* and ecologically the area appears more typical of one inhabited by *C. douglassii.*

SPECIES DISCUSSION

Cardamine bulbosa is an extremely variable species in comparison to *C. douglassii.* This may be seen morphologically through the use of correlation and distance coefficients and with the Davidson and Dunn (1967) i value. It has also been shown that both chemically and ecologically *C. douglassii* is much less variable than *C. bulbosa.* If the assumption of an increase in variability through time is correct, *C. douglassii* is probably derived from the widespread *C. bulbosa.*

Three chemical races of *Cardamine bulbosa* were found. *Cardamine bulbosa* forma *fontinalis,* found growing almost submerged in cold spring water, possesses one of the distinctive chromatographic patterns (Race B). The other two chemical races do not differ ecologically. However, one (Race C) normally has more branches and tends to have white to slightly pink flowers and a large amount of pubescence. Race A normally has few, if any, branches, and white flowers with only a small amount of short pubescence. Stuckey (1962) considered naming the varieties he found which differed as to the amount of sepal pubescence. His pubescent variety appears to be equivalent to chemical race C. Populations of this chemical race can be identified morphologically; however, identification of many individual specimens without chromatograming is questionable. Based on the numerous branches and hairs it would appear that the type specimen (Type *Clayton s. n.* BM, ex. Herb. Gronovii) of *C. bulbosa* belongs to chemical race C.

In *Cardamine bulbosa*, chemical race C possessing quercetin and chemical race A hybridize easily under greenhouse conditions. It is also quite difficult to tell some of these individuals apart based on external morphological characters. Further investigation into the genetics and geographic distribution of the chromatographic patterns may indicate the biological and evolutionary importance of the different patterns and their associated morphological characters. If further research indicates that *C. bulbosa* — chemical race A tends to be similar in range to *C. douglassii*, then a close look at the morphology of *C. douglassii* and the various races of *C. bulbosa* will be necessary. In number of branches, petal length, as well as the chromatographic pattern itself, *C. bulbosa* — chemical race A is more similar to *C. douglassii* than it is to *C. bulbosa* — chemical race C. The possibility that *C. bulbosa* — chemical race A is a variety of *C. bulbosa* possessing some genetic material from *C. douglassii* should not be overlooked in further investigations.

The evolutionary significance and the geographic distribution of polyploidy within *Cardamine bulbosa* and *C. douglassii* is poorly understood. The presence of the chromosome number of $n = 48$ in the two northern populations of *C. douglassii* studied may be explained by the fusion of a normal haploid egg ($n = 32$) with an unreduced diploid sperm. The fact that some plants with haploid numbers of 72 in addition to plants with $n = 48$ were found in population 15 at Bedford, Ohio, lends further support to this hypothesis.

Low intrapopulational variability, as indicated by high Davidson and Dunn i values, is found in ecologically borderline habitats of both species. The highest i values were found in a very dry community containing *C. bulbosa* and a wet, disturbed community containing *C. douglassii*.

The logic of recognizing two species, *Cardamine bulbosa* and *C. douglassii*, was previously questioned by Schulz (1903). On the other hand, most modern authors have recognized these taxa as two species.

Before resolving this conflict, one must define his own species concept. Mayr's (1969) biological species definition states that "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups." Many botanists prefer different species to have a "certain degree" of morphological difference in addition to reproductive isolation. This is due, in part, to the presence of polyploidy and apomixis in plants, as well as the opinion of some that a classification should be useful to the herbarium or classical taxonomist.

Isolation of the genomes is extremely important in the development of biological species. Assume, for instance, that *Cardamine bulbosa* contains genes which help adapt the species to one particular niche. These particular genes are present in the individual breeding population because of selection by the environment. A second breeding population (*C. douglassii*) in a different ecological niche but in the same community as the first is exposed to different selective forces and would contain different arrays of co-adapted genes.

Crossing between members of the different populations will produce many untested presumably poorly adapted genotypes. If the parental populations differ with respect to an adaptive gene combination of ten or more independent genes, over 99.9% of their F_2 zygotes could be expected to be subvital recombinations (Grant, 1971).

This huge loss of reproductive potential resulting from interbreeding of differentiated sympatric populations can be contrasted with the generally beneficial results of interbreeding within the same population. In this situation blocks to hybridization would be favored by natural selection and would spread through each of the populations (Grant, 1971; Wallace, 1968).

A stable biotic community is composed of reproductively isolated breeding populations. Sexual reproduction between members of different breeding populations would lead to the breakup of adaptive gene combinations within each population. A biological species, then, is composed of breed-

ing populations reproductively isolated in nature. This reproductive isolation must be somewhat complete in order to prevent the breakup of beneficial gene combinations. The isolation mechanisms necessary for two lines to evolve separately may be internal or external and need not act under laboratory conditions or in artificial cross pollinations.

Sokal and Crovello (1970) conclude that the biological species definition will not apply equally to both the classical species (morphological) and the evolutionary species. Furthermore, in effect, two or three species definitions may be required.

The particular species definition used in any one group of plants may depend in part on the plants themselves as well as the background or emphasis of the particular taxonomist. Grant (1963, 1971) and Cain (1954) recognize the importance and need of distinguishing between the biological species and the taxonomic species. The biological species definition emphasizes reproductive isolation while the taxonomic species definition emphasizes phenetic or morphologic variation. Both are commonly related to each other; however, many problems arise from sibling species, polyploidy and apomixis.

An emphasis on evolution instead of reproduction or morphology necessitates a third species definition, that of the evolutionary species (Grant, 1971). The evolutionary species is a group of populations which are 1) an ancestral-descendant sequence of populations, 2) evolving separately from other such lineages, 3) fitting their own particular ecological niche in a biotic community, 4) susceptible to change in their evolutionary role through time (Simpson, 1961).

Much of the argument over species definition appears to depend on the background of the taxonomist. At times it may be necessary to discuss the classification of a population of plants from these three viewpoints — biological, taxonomic, and evolutionary. "Viewed from the standpoint of the evolutionary species concept, however, the

important question is not whether two species hybridize, but whether two hybridizing species do or do not lose their distinct ecological and evolutionary roles. If, despite some hybridization, they do not merge, then they remain separate species in the evolutionary perspective" (Simpson, 1961).

Considering the total biology of *Cardamine bulbosa* and *C. douglassii* permits a better understanding of the species problem in these two taxa. In the study of *C. bulbosa* and *C. douglassii*, artificial hybrids were produced with relative ease in the greenhouse. In addition, natural putative hybrids were found in three different areas in Ohio. Since these two taxa are not completely reproductively isolated, they cannot be considered as different biological species.

The status of *Cardamine bulbosa* and *C. douglassii* as a taxonomic species is dependent on the amount of morphological difference one equates with species differences. The data previously presented indicate that both taxa can always be separated on pubescence length assuming little or no hybridization is occurring in the population. Other good characters separating the taxa are: flowering time, habitat, flower color, height, and time of meiosis. Taking into account both the morphological and physiological characters studied, one can always differentiate between so-called good *C. bulbosa* and *C. douglassii*. As the characters separating the two species appear to be fixed and are not variable from place to place one must assume that the taxa are genetically isolated from one another. They are not completely isolated by pollinator activity, crossing behavior, phenology or ecology. However, as each of these isolation mechanisms is nearly complete, one would expect that the two species are very close to being completely isolated from one another in nature. In areas, particularly along the northern edge of their ranges, where the isolation mechanisms break down one may find hybrid swarms and introgression to *Cardamine bulbosa;* however, the evolutionary importance of this is unknown. Man has probably been instrumental in the formation of these swarms

through farming techniques and various kinds of disturbances. He has similarly kept these swarms from interacting evolutionarily by isolating one population from another. It seems, then, that one may call these two taxa good taxonomic and evolutionary species as they are separated by reasonably good morphological characters and are presently distinct evolutionarily. This does not mean that future gene flow could not cause species breakdown.

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